U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 228.
B. T. GALLOWAY, *Chief of Bureau.*

THE HISTORY AND CAUSE OF THE COCONUT BUD-ROT.

BY

JOHN R. JOHNSTON,
Assistant Pathologist, Laboratory of Plant Pathology.

Issued February 5, 1912.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1912.

22900273345

Med
K4961

DISEASED COCONUT TREE, SHOWING ONE SPIKE (AT "X") THAT HAS LOST ITS NUTS. ALL THE OTHER SPIKES ARE HEAVILY LOADED AND TOGETHER BEAR ABOUT 130 NUTS.

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 228.

B. T. GALLOWAY, *Chief of Bureau.*

THE HISTORY AND CAUSE OF THE COCONUT BUD-ROT.

BY

JOHN R. JOHNSTON,

Assistant Pathologist, Laboratory of Plant Pathology.

Issued February 5, 1912.

WASHINGTON:

GOVERNMENT PRINTING OFFICE.

1912.

BUREAU OF PLANT INDUSTRY.

Chief of Bureau, Beverly T. Galloway.
Assistant Chief of Bureau, William A. Taylor.
Editor, J. E. Rockwell.
Chief Clerk, James E. Jones.

Laboratory of Plant Pathology.

SCIENTIFIC STAFF.

Erwin F. Smith, *Pathologist in Charge.*

R. E. B. McKenny, *Special Agent.*
Florence Hedges, *Assistant Pathologist.*
Nellie A. Brown, Lucia McCulloch, and Mary Katherine Bryan, *Scientific Assistants.*

228
2

22778328

WELLCOME INSTITUTE LIBRARY
Coll. welMOmec
Call No. QK

LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF PLANT INDUSTRY,
OFFICE OF THE CHIEF,
Washington, D. C., June 22, 1911.

SIR: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 228 of the series of this Bureau the accompanying technical paper by Mr. John R. Johnston, entitled "The History and Cause of the Coconut Bud-Rot."

This paper deals with a very destructive and widespread disease of coconuts which has been known to occur in Cuba for more than 30 years, and undoubtedly the same disease occurs also in Jamaica, in the Cayman Islands, in British Guiana, and in British Honduras.

The results presented are based on investigations covering a period of four years, the extent and nature of the disease having been studied in Cuba, Jamaica, Trinidad, and British Guiana.

Respectfully,

B. T. GALLOWAY,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

CONTENTS.

228

ILLUSTRATIONS.

PLATES.

TEXT FIGURES.

B. P. I.—691.

THE HISTORY AND CAUSE OF THE COCONUT BUD-ROT.

INTRODUCTION.

For more than 30 years the people of Cuba have discussed the cause of the gradual dying off of their coconut trees and have tte mpted to overcome it, but without success. As a result of the unchecked progress of the disease the coconut groves have now almost disappeared from the western part of the island and are confined in a commercial way to a very small strip along the coast in the Baracoa district at the extreme eastern end. Ten to eighteen million nuts have been exported from this locality to the United States annually for the last few years. Dr. Erwin F. Smith, working on the disease in 1904, in the neighborhood of Baracoa, writes as follows: "If it continues to spread as it has done during the past 10 years it will inevitably destroy the coconut industry of the island, and that, too, within the next 10 or 15 years." [1]

This disease of the coconut is by no means confined to Cuba. It has caused great loss in Jamaica, British Honduras, Trinidad, and British Guiana, countries that are important sources of coconuts for the United States. The trouble occurs also in less important places in tropical America. A dying off of coconut trees in the Eastern Hemisphere is thought by some to be caused by a disease identical with that in the West Indies. It is probable that this is a widespread trouble, occurring wherever coconuts are grown. Desultory studies have been made of this disease at intervals ever since the early eighties, and it has been ascribed to various causes, such as insects, fungi, bacteria, atmospheric conditions, and soil. A malady so actively destructive in certain districts demands more attention from scientific investigators.

The present work has been carried on with the hope of establishing the cause and finding a remedy. The writer believes he has succeeded in showing that the disease is infectious and that it is due to

[1] Smith, Erwin F. The Bud Rot of the Coconut Palm in the West Indies. Science, n. s., vol. 21, Mar. 31, 1905, pp. 500-502.

228

certain specific bacteria, but methods by which it can be absolutely controlled remain yet to be found. A thorough knowledge of the conditions under which the disease occurs, including the difficulties involved in carrying on an investigation of it, is so important that it has been considered desirable to describe in some detail the work carried out by the writer. The salient points brought out by the observations of earlier investigators have also been included.

NATURE OF THE DISEASE.

General diagnosis.—The common name of the disease, bud-rot, well describes its nature, for in its acute or advanced stages the bud of the tree, i. e., the growing point in the center of the crown, is affected by a vile-smelling soft rot which destroys all the younger tissues. At this stage most of the nuts have fallen, the lower leaves are turning yellow, and the middle folded and undeveloped leaves are dead and hang down between the still green surrounding leaves. Signs of the disease in its incipiency are (1) the falling of the immature nuts (Pl. I); (2) a staining of the opening flower spikes, partly or wholly, to a rich chocolate brown (Pl. II, figs. 1 and 2; and Pl. III, fig. 1); and (3) the dying and bending over of the middle undeveloped leaves. When the nuts are being shed investigation reveals at the base of the affected spikes a dark-colored wet rot which spreads around the leaf sheaths, or strainers, as they are locally known. This rot appears as water-soaked areas which may reach a length of 15 or 20 centimeters on both the upper and lower surfaces of the bases of the leaves (Pl. II, fig. 3; and Pl. III, figs. 2 and 3). This condition often penetrates the leaf bases to a depth of 2 centimeters or more, and the tissues involved in it swarm with bacteria. As the white tissues at the base of the leaf become old and green the water-soaked spots harden, and they may often be found in this condition on otherwise perfectly healthy trees.

The rot gradually spreads from the base of one spike to another through the wet strainer. It is probable that insects carry the disease from one part to another, since there may be one or more points of infection. Gradually all the spikes become affected and shed their nuts, and the leafstalks become so rotted at their bases that they are not able to maintain their natural position, but are pendent (Pl. IV), often for a long time, or else fall off.

If the infection starts in the central leaves the disease is apt to progress rapidly downward into the younger tissues, which it is very active in disintegrating, the vascular bundles being so soft as to allow the tissues to go entirely to pieces. In the center it may progress into the trunk for a short distance and rot out the fundamental tissue,

FIG. 1.—OPEN FLOWER SPIKE OF COCONUT PALM WITH DISEASED, BLACKENED TIPS. FIG. 2.—SAME, MORE ADVANCED, WILTED. FIG. 3.—WATER-SOAKED SPOTS ON INSIDE OF PETIOLE; HEALTHY SWORD AT BASE.

leaving only the fibers which are too hard to be disintegrated. This rot has been found, exceptionally, as far as 1.5 meters under the heart of the bud, a hard outer shell being left around the central rotted portion. Usually the decay extends in the trunk under the bud for a distance of only 0.2 to 0.5 meter and never throughout its length.

Spots which are merely fungous infections often occur on the middle leaves (Pl. V, figs. 1, 2, and 3). These spots spread and coalesce, leaving blackened, wet, and later, dry and dead tissues. Insects and small animals are often found in the decaying tissues, but the advancing margin of the soft rot appears to be occupied exclusively by bacteria.

Spread and loss.—The spread of this disease may be very rapid. It may occur year after year as only scattered cases in a grove, but frequently whole plantations may be affected in a short time. In such groves scores and scores of bare trunks may be seen (Pl. VI, fig. 1), the crowns of which have rotted and blown off. There may be trees with the whole crown bent over and hanging downward (Pl. VI, fig. 2), and others with three or four ragged leaves waving upright in the air and all the rest brown, broken, hanging down, and dead (Pl. VII). In the midst of this desolation there are often some green-crowned trees retaining a few nuts, or still in good bearing. From two months to more than a year may elapse from the time of the infection of a tree to its destruction. In Cuba a certain grove of 450 trees was totally destroyed in two years. Another grove was reduced from 1,200 to 300 bearing trees in the same time. A planter in Jamaica who formerly obtained a revenue of £5,000 per year from his coconuts now gets barely £500. Of an estate in Trinidad comprising some 5,000 trees only 15 per cent are standing at present (1907). Formerly many coconuts were grown on the Grand Cayman Island, but the industry has now been wiped out. In fact nearly every coconut-growing region of importance in the West Indies has been invaded by this menace to the industry.

GENERAL DISTRIBUTION OF THE DISEASE.

The coconut bud-rot has been studied most carefully in the West Indies. It has been reported from various parts of the Eastern Hemisphere and probably occurs in all tropical lands.

TROPICAL AMERICA.

Cuba.—While coconuts are grown in suitable places all over Cuba, coconut growing on a commercial scale is now mostly confined to a narrow strip of land on the north shore at the extreme eastern end

of the island (fig. 1). This strip, which is about 80 kilometers long, is mostly within what is known as the Baracoa district. The bud-rot has been reported at La Gloria;[1] it occurs from Havana to Artemisa, at Cardenas, Cienfuegos, Manzanilla, Banes; on the coast between Santiago de Cuba and Cape Cruz;[2] and from Cape Maisi northwest to beyond Baracao. All the trees have been killed at the extreme eastern end of the latter strip of land and largely about Baracoa and in other more isolated places. The estimated monthly loss to the Cuban industry is $10,000.[3] The fact that coconuts are not now grown commercially over the greater part of the north shore of the island,

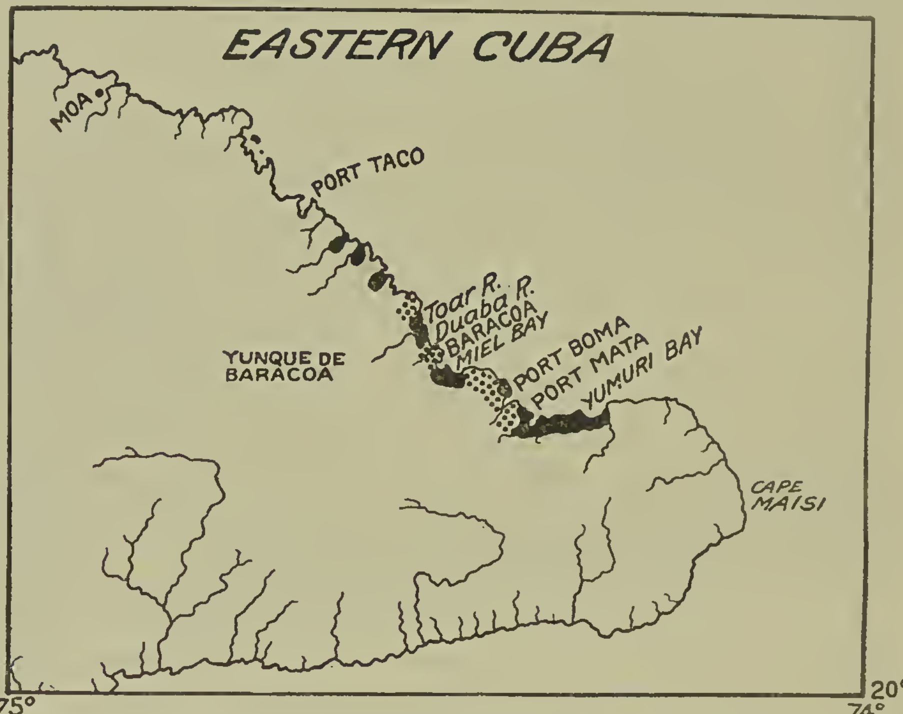


FIG. 1.—Map of the eastern end of Cuba, showing the location of coconut groves (dots). The diseased areas are indicated by heavy shading.

a distance of 900 to 1,100 kilometers, is attributed by some to the supposed prevalence of this disease in early times in those regions.

Diseases of the coconut palm have been reported from various parts of the West Indies for some years. In many cases the descriptions are so meager that it is impossible to identify them with the bud-rot, nevertheless the one characteristic, the rot in the heart tissues, is believed to apply only to this disease. In addition, the dying of the central undeveloped leaves is taken as a sign of the bud-rot, as it is usually the result of the rotting of the lower tissues.

[1] Merrick, F. Cocoanut Bud Rot. Cuba Review, vol. 6, April, 1908, p. 24.

[2] Horne, W. T. Bulletin No. 15, Estación Central Agronómica de Cuba, July, 1908, p. 4.

[3] Horne, Mary Tracy (Mrs. W. T. Horne). The Cocoanut Industry in Cuba. Cuba Review, vol. 5, no. 11, October, 1907, pp. 18–20.

With these considerations as a basis for their selection and as preliminary to the writer's own observations, the following extracts from earlier writers are made, and in order to avoid misinterpretation the exact statements are included.

Dr. Federico Galvez, in a letter[1] dated Havana, January 5, 1886, writes that when he returned to Cuba after an absence of more than 10 years, the place of his birth and childhood, Matanzas, presented a very different appearance in that all of the once beautiful coconut trees had been completely destroyed. Thousands of these dead trees were seen by him. The same conditions prevailed in Havana. Of the extensive coconut groves of Marianao and Jesus del Monte only a few isolated cases now remained standing. In his exact words:

> Cuando volví á Cuba despues de una ausencia de más de diez años, fuí á visitar los campos donde había pasado mi niñez, y tambien á mi ciudad natal, Matanzas, y tanto en esta como en aquellos, me impresionó dolorosamente ver todos los cocoteros que tan frondosos había dejado, completamente destruidos. * * *
>
> En los alrededores de la Habana sucedía lo mismo; de los extensos cocales de Marianao y Jesús del Monte solo quedaban en pié algunos árboles aislados. * * * Miles de cocoteros muertos durante este tiempo han sido examinados.

Sr. Antonio Bachiller, in a letter[2] dated Havana, January 26, 1886, stated that he had examined many trees dead and dying in several towns near Havana, and in none of the trees did he find the insect, or any sign of it, which was said to cause the trouble. He did find signs of putrefaction in the crown. To quote exactly:

> He hecho abrir en Guanabacoa, en Marianao, en Cimarrones, en Camarioca, muchos árboles ya muertos ó en estado de morirse, y en ninguno se ha encontrado la larva del supuesto cucarachon, ni sus huellas ó galerías. * * * Solo en el penacho había señales de putrefacción con sus consequencias: allí he hecho recojer hidrófilos comunes, agua fétida y cucarachas.

One other letter from among the numerous ones published at this time is selected for reference. Raphael del Pino, in a letter[3] dated Hacienda Herradura, Pinar del Rio, January 25, 1886, says that he lost on his plantation more than 100 trees, all small ones from 1 to 1½ years old. To quote from him:

> He perdido más de cien matas en esta hacienda. * * * Esas cien matas de coco eran todas pequeñas, de un año á año y medio las que más edad tenían.

From these letters, and many that have not been quoted, it is evident that a serious disease of coconuts has been present in Cuba for many years, according to Dr. Federico Galvez, at least some years prior to 1886. From Bachiller's mention of putrefaction it is more

[1] Balmaseda, F. J. Tesoro del Agricultor Cubano, vol. 2, 2d ed., 1893, p. 154.

[2] Balmaseda, F. J. Op. cit., p. 132.

[3] Pino, Raphael del. El Pais, Jan. 29, 1886. Reprinted by Balmaseda, F. J., in Tesoro del Agricultor Cubano, vol. 2, 2d ed., 1893, p. 135.

than likely that the disease was no other than the bud-rot, and from the fact that the notes of Galvez and of Pino apply to adjacent districts it may reasonably be supposed that they were speaking of the same disease. More recent investigations by Mr. August Busck and Dr. Erwin F. Smith, both of this Department, and a little later by the staff of the Estación Agronómica at Santiago de las Vegas, deal with the present occurrence of the disease. Mr. Busck,[1] in 1901, reported as follows on the disease in the Baracoa district:

> There were no diseased palms in the immediate neighborhood of Baracoa, but going out some 10 miles east and along the coast, yellow, drooping tops and naked trunks began to appear; and still farther out around Mata and neighboring towns, the disease reached its highest development. Here large areas were attacked, and already from 10 to nearly 100 per cent of the trees were lost.

Dr. Smith studied the disease in 1904 and reported as follows:

> The disease has made decided advances since it was studied by Mr. Busck in 1901, especially at Mata, and if it continues to spread as it has done during the past 10 years it will inevitably destroy the coconut industry of the island, and that, too, within the next 10 or 15 years. Already many of the planters are discouraged and are not setting any more trees, since it now attacks trees of all ages, including quite young ones, and those on the hills as well as those close to the sea.[2]

In their papers on the subject Mr. Busck and Dr. Smith describe the nature of the disease in such detail as to render it certain that it was the bud-rot which they were studying.

In the Primer Informe Anual de la Estación Central Agronómica de Cuba, 1905, on page 195, there appears the following:

> Esta enfermedad se presenta en la Provincia de la Habana, y se nos ha dado cuenta de que existe en otros varias localidades, probablemente afecta á toda la Isla.

In this quotation there is no direct mention of the bud-rot, but further on in the article the disease is described as the bud-rot identical with that in eastern Cuba. According to this evidence the disease is now present in the Province of Havana.

A former pathologist of the Estación Central Agonómica and the writer have carried on investigations more recently, and their work will be discussed more fully further on.

Jamaica.—In Jamaica the coconut region is proportionately more extensive than in Cuba, the only districts where there are no large groves being in the interior and on the south coast (fig. 2). Fortunately, the disease is serious only in the extreme western end of the island, in the district between Savanna la Mar and Montego Bay and a little beyond. It is not greatly feared by those planters who keep watch of their groves, although even with the utmost care many lose

---

[1] Busck, August. Report on the Investigations of Diseased Cocoanut Palms in Cuba. Bulletin 38, n. s., Bureau of Entomology, U. S. Dept. of Agriculture, 1902, pp. 20–23.

[2] Smith, Erwin F. The Bud Rot of the Coconut Palm in the West Indies. Science, n. s., vol. 21, 1905, pp. 500–502.

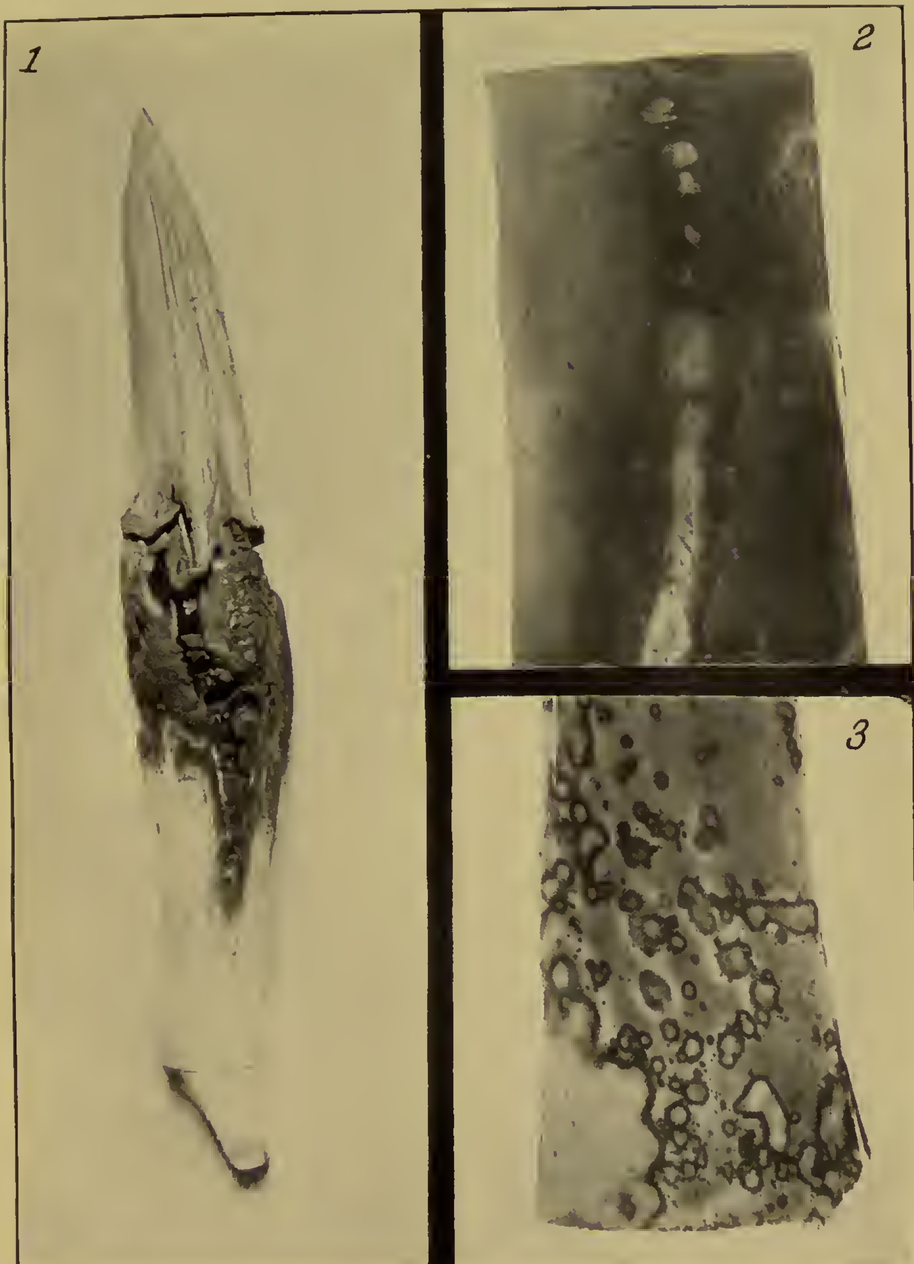


FIG. 1.—ROTTED SWORD OF COCONUT PALM. FIGS. 2 AND 3.—WATER-SOAKED SPOTS ON INSIDE AT BASE OF PETIOLE.

a dozen or so trees every year. The total loss for Jamaica per year at present is probably small, but the fact that the bud-rot occurs there and requires constant watching indicates a dangerous condition.

Mr. W. Fawcett, former director of the Botanical Gardens at Kingston, reports as follows:

I have visited Montego Bay to examine into the death on a large scale of coconut palms in that neighborhood. * * * Several trees were cut down and the roots, stem, leaves, and cabbage examined. There was no evidence whatever of attacks by a beetle. There were some small larvæ, some wood lice, earwigs, ants of several species, and other insects on the affected parts, but they were evidently only preying on the diseased juices, and were not the cause of the disease. * * *

The youngest parts were those affected. The leaves and flowers in the bud were sometimes able, though affected, to withstand the disease so far as to open out, and some leaves and nuts attained almost their full development before the tree succumbed. In the case of tall trees the first indication of the disease was the dropping of the young fruit. * * *

If the terminal bud in the cabbage is affected, the tree is doomed.

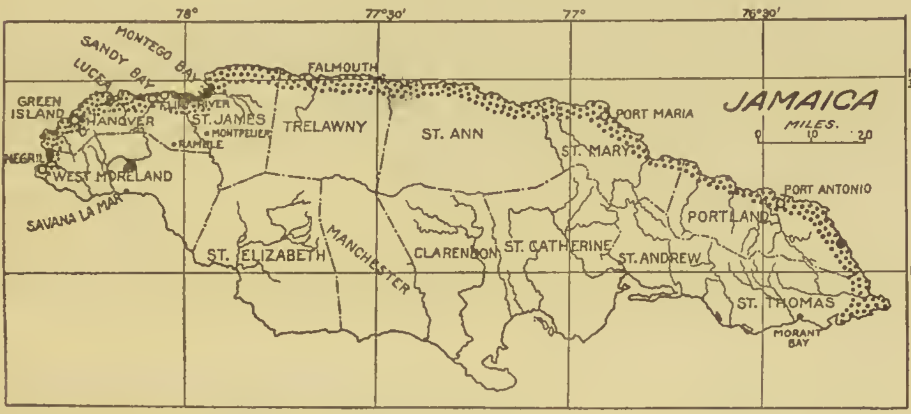


FIG. 2.—Map of Jamaica. The dots show the location of coconut groves, and the heavily shaded portions indicate diseased areas.

In almost all the trees examined the sour smell of a putrefactive fermentation was very noticeable, and I am of the opinion that the disease is due to an organized ferment which is able to attack the very tender tissues of the youngest parts, even outside the terminal bud. If this ferment can be destroyed by fire or other means before it reaches the terminal bud in the heart of the cabbage the tree may be saved.[1]

Cayman Islands.—In the Cayman Islands, midway between Jamaica and Cuba, the bud-rot has raged for some time. The industry has been practically destroyed on Grand Cayman. Mr. W. Fawcett reports:

Disease has for several years blighted the palms in Grand Cayman. * * * No accurate information could be obtained from the people as to the first appearance of the disease; some said it was 15 years ago, others, again, thought it might have been 40 years. In a dispatch from the Marquis of Sligo in 1834 he mentions that all the coconuts of the leeward side had been destroyed, but that the infection had not reached

[1] Fawcett, W. Report on the Coco-nut Disease at Montego Bay. Bulletin 23, Botanical Department of Jamaica, September, 1891, p. 2.

the windward side. It is probable that this was the same deadly disease. I saw a great number of these palms of different ages in various stages of the disease, and at several localities. * * * The inhabitants have been most persevering in their efforts to reestablish their coconut walks, but it is of no avail.[1]

Mr. Fawcett also describes the disease in full as like that of Jamaica, which, in turn, is similar to that of Cuba.

British Honduras.—A disease not due to insects has been noticed on the coconut trees of Honduras for some years. It has been reported as follows:

It is known as "fever," and at present no accurate account has been given of its symptoms or of its prevalence. * * * From the little known about it, it appears to be allied to one or other of the diseases (if, indeed, they are not the same) observed in Demerara in 1875–6, and in Montego Bay, Jamaica, in 1891. * * * According to Mr. Hunter, 50 to 80 per cent of the trees attacked by the weevil show signs of the disease at the top first. This may be merely a misinterpretation of the early signs of injury due to weevil grubs before they have been noticed in the trunk, but the statement is of importance and should be confirmed or refuted. In his evidence Mr. Baber says he "has a small spot on the seaside in Serango Bight (very swampy). He there noticed that the trees died off very rapidly, although of various ages from 7 to 10 years. Does not know the cause of death; some trees on better land close by were not affected." Mr. Schofield states that his plantation was apparently healthy on the 24th of December. * * * On the 7th of January he discovered some 15 trees more or less affected; some had actually fallen over, others had their fronds broken and trailing on the ground, while the rest from their yellow and drooping appearance showed plainly that they also were diseased.[2]

The following extract is from a letter to the United States Department of Agriculture from Belize, British Honduras, dated April 12, 1907:

We have in this colony thousands of trees killed every year either by insects, bacteria, or a combination of both.

These reports from British Honduras indicate that the disease referred to can scarcely be any other than the bud-rot.

Trinidad.—In Trinidad a disease occurs along the west coast (fig. 3) and in the interior, leaving the extensive groves of the east coast untouched.

Mr. J. H. Hart, formerly superintendent of the Botanical Gardens, says:

My observations lead me to conclude that the plantation itself affords distinct evidence that there has been for many years a succession of deaths among the trees on certain areas, which latter appear to have been replanted several times over. In my opinion this is strong evidence that the disease is not new but has been present in more or less severity for years.[3]

---

[1] Fawcett, W. Report on the Cayman Islands. Bulletin 11, Botanical Department of Jamaica, February, 1889, pp. 3–4.

[2] Blandford, W. H. Palm Weevil in British Honduras. Kew Bulletin, Nos. 74 and 75, 1893, pp. 27–60.

[3] Hart, J. H. Bud-Rot Disease in Coconuts, Gulf Coast, 1905. Preliminary Report. Bulletin of Miscellaneous Information, Botanical Department of Trinidad, October, 1905, pp. 242–243.

Mr. F. A. Stockdale does not appear to consider the disease serious:

The few isolated cases in the Cedros district would indicate that this disease is not of a very infectious character, but large numbers have been killed out in the Siparia district, the spread being very rapid and apparently from the windward. I am inclined to the view that this diseaso is similar to the destructivo disease of cocoanuts in Cuba.[1]

There has been a good deal published on the coconut-palm disease of Trinidad, and while the early local investigators admitted the presence of some bud-rot, they maintained that the worst of the injury was due to other diseases. The description and arguments of the various writers appear so unsatisfactory that they will be discussed more fully in a later paragraph. It is the belief of the writer from

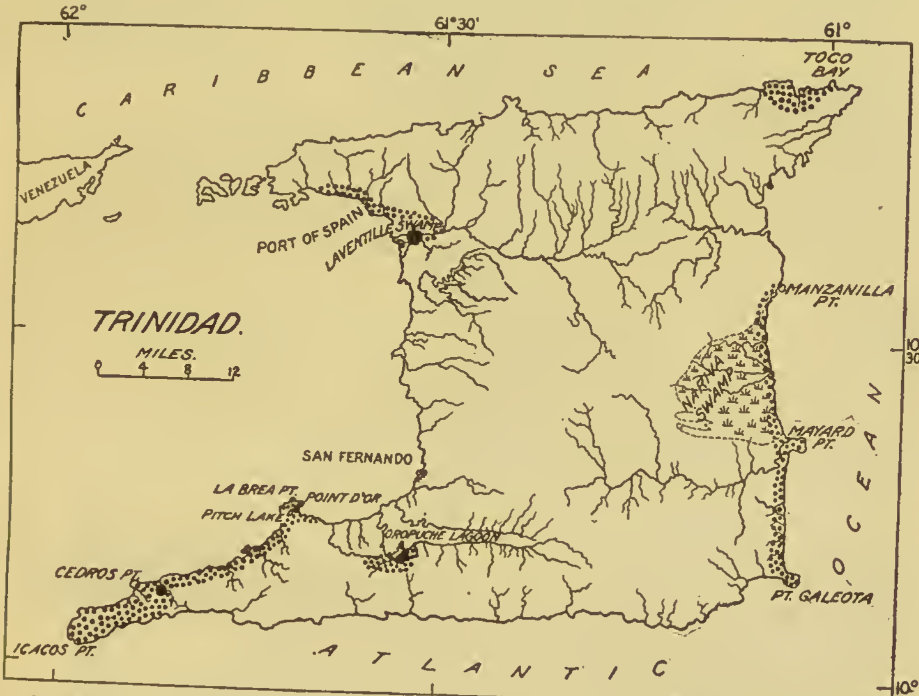


FIG. 3.—Map of Trinidad. The location of coconut groves is shown by dots. The heavily shaded portions indicate diseased areas.

personal examination in many places in the island that the bud-rot is the principal disease in Trinidad, and that the others are of less importance, or represent stages secondary to the bud-rot.

British Guiana.—In British Guiana the groves at the mouths of the Essequibo and Mahaicony Rivers are diseased (fig. 4). Hon. William Russell examined the trees and reported, in correspondence to the Kew Gardens in 1875, as follows:

On dissecting the top of the tree, all the fruit germs wero found quite rotten (putrid fermentation), and gave a most offensive smell; and at the point where the last frond or central spike divides from the lower fronds the stato of putrefaction was fearful.[2]

[1] Stockdale, F. A. Coconut Palm Disease (Society paper 247). Proceedings of the Agricultural Society of Trinidad and Tobago, vol. 7, December, 1906, p. 45.

[2] Anonymous. Bud-Rot Disease of Cocoanut Palm. West Indian Bulletin, vol. 6, 1905, pp. 307-321.

From this same colony comes the following report:

Travelers on the East Coast Railway can hardly have failed to notice the unhealthy appearance of many of the coconut trees which form so conspicuous a feature of the district from Mahaicony onwards to Belladrum. The drooping leaves, and yellow crowns, the "bare poles" of dead palms in too many cases point to disease of a widespread and malignant nature.[1]

So far as authentic reports or personal investigations are concerned there is no note of the further occurrence of the disease in the West Indies or tropical America. Three different travelers have, however, reported to the writer a disease of coconuts in both Haiti and the Dominican Republic similar in general aspect to the Cuban disease. Another traveler reports it from the Mexican coast south of Vera Cruz. A captain of a schooner engaged in collecting coconuts at Baracoa

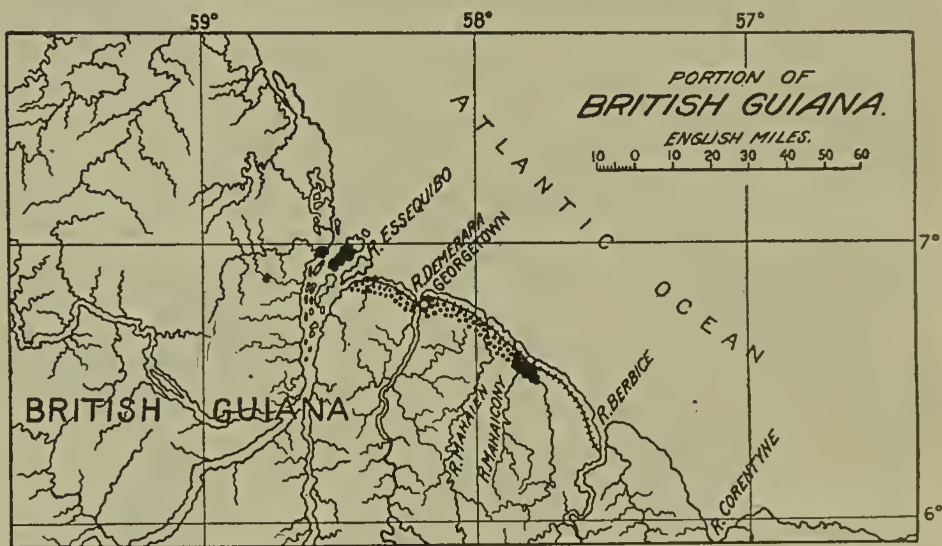


FIG. 4.—Map of a portion of British Guiana. The dots show the location of coconut groves and the heavily shaded parts indicate diseased areas.

claimed to the writer that he had seen what appeared to be the same disease on the San Blas coast of Panama, where the best nuts are obtained.

Although it does not appear to occur in Porto Rico, it is prevalent in all of the very important sources of coconuts in the West Indies and the adjacent coasts.

EASTERN TROPICS.

Diseases of coconuts in the Eastern Hemisphere have been known and investigated for some years, but it is only recently that one similar to the bud-rot of the West Indies has been reported. So far as known there have been no comparisons by photographs or by

[1] Leechman, Alleyne. The Radical Cure of Infectious Plant Diseases. Journal of the Board of Agriculture of British Guiana, vol. 2, no. 3, January, 1909, pp. 104–106.

DISEASED COCONUT TREE AT MONTEGO BAY, JAMAICA.

specimens of the bud-rot from both regions, nor has any investigator of the one region visited the other, a procedure eminently desirable to establish fully the probable fact that this destructive disease is present in all parts of the tropical world.

Philippine Islands.—In the Philippines the disease is present in several provinces and reported [1] to be very destructive.

> The bud-rot is at present very prevalent in Lazaan, Sungi, and Ylaya. It is present, but does less damage, in two other of the upper barrios of Lilio. There are a few scattered cases in Balanacan and Sinipian, barrios of Nagcarlan, and probably in Pagsauitan and elsewhere. * * * Capt. Grove has heard that many years ago it practically wiped out the coconut industry of Lucban, and I have been told that it was very destructive about five years ago in Sariaya. * * *
>
> In the badly infested districts there are patches where almost every tree is smitten and larger ones where fully half of the trees are dead or dying. * * *
>
> The disease attacks the soft, undifferentiated tissue of growing points. * * * As soon as the youngest leaf is noticeably discolored it can easily be drawn out. * * * The decaying tissue has a powerful and vile odor. The stench is very characteristic.

Ceylon.—In Ceylon an infection apparently identical with the bud-rot of the West Indies destroyed the young trees on a small estate but spread no farther. An investigator reported that bud-rot, apparently identical with the West Indian disease, appeared in a small native estate early in the year. The place was visited and the diseased trees cut out.[2]

According to Copeland,[3] Petch of Ceylon found in a small isolated patch of 10 acres, including some 800 trees, 50 that were dead or dying. The diseased trees were 3 or 4 years old. Their condition is described as follows:

> The first indication of the disease (in the case of young plants) is the withering of the youngest unfolding leaf. This turns brown and can be pulled out of its sheath; it is then found to end in a soft brown mass. * * * If the dying fronds are removed and the bud exposed there will be found instead of the white cabbage a pale brown semiliquid mass. * * * The organisms responsible for this decay are bacteria which are found in abundance in the rotting tissues; they are short, thick rods with rounded ends which form whitish colonies of slow growth on sugar agar.

India.—It is probable that the bud-rot occurs also in India proper, according to the following:

> Some time ago the occurrence was reported of a coconut pest in the shape of a fungus which was eating into the vitals of the coconut palm in North Travancore.[4]

[1] Copeland, E. B. Bud rot of the Coconut. Philippine Agricultural Review, vol. 1, no. 5, May, 1908, pp. 210–220.

[2] Hart, J. H. Diseases of Cacao, Coconut, Rubber, etc. Extract from the Report of the Botanical Department, Ceylon (Society paper 264). Proceedings of the Agricultural Society of Trinidad and Tobago, vol. 7, 1907, pp. 179–193.

[3] Copeland, E. B. Op. cit., pp. 210–220.

[4] See "Fungus Disease of Coconuts," in Tropical Agriculturist, vol. 24, February, 1905, p. 556.

A report of palm diseases in India mentions the coconut palm as follows:

> The most serious aspect of the matter is the fact that coconut palms are undoubtedly subject to infection. In Ramachandrapuram taluka few cases only were seen, but in Amalpuram they are numerous, though fewer than in the palmyra. * * * In one locality somo 200 dead coconut trees were seen; elsewhere only a dozen or two. The danger is that the disease may increase in virulence in regard to coconut palms if allowed to rago unchecked. * * * Very soon a rot follows, which extends with great rapidity in the delicate central tissues and converts the whole heart into a foul-smelling mass of putrefaction in which everything is involved, and the original agent is lost sight of.[1]

German East Africa.—In German East Africa a disease of coconuts is described as a rot of the heart tissues and is said to be contagious. The symptoms are merely that the leaves turn yellow and dry up, and the tree dies. Soon after the first appearance of the disease the heart leaves can be drawn out, as the bottom is rotted off. This meager description of it answers well for the typical bud-rot:

> Die Fäulnis des Herzblattes is weit schlimmer, da sie ansteckend ist. Die Krankheit macht sich folgendermassen bemerkbar: Die unteren Wedel und die Spitze des Herzblattes werden gelblichrot und trocken, und der Baum stirbt ab. Man kann nach der ersten Erscheinung das Herzblatt mit leichter Mühe herausziehen, da das Ende vollkommen verfault ist. Ist das Herz verfault, so sind die Wurzeln und auch noch der untere Stamm vollkommen frisch und saftig, ein Zeichen, dass die Krankheit nicht von unten an den Wurzeln anfängt, wie leider hier noch vielfach behauptet wird.[2]

Portuguese East Africa.—In Portuguese East Africa a similar disease is reported, and there is great probability that it is the bud-rot:

> In Quilimane the disease attacks the leaves, which become discolored and dried without there being any insect pest or any visible disease present. The disease quickly spreads from tree to tree until a whole plantation is destroyed. In Quilimane the only remedy known is the total destruction of the diseased tree in an early stage of the disease by cutting down and burning.[3]

Tahiti.—Since the manuscript of this bulletin was prepared report has been received by this Department through the Secretary of State of a serious disease of the coconut palm in Tahiti suspected to be identical with the West Indian disease.

From the foregoing it will be apparent that the bud-rot of the coconut is probably present in all parts of the tropical world (fig. 5). That it is such a cosmopolitan disease makes it doubly important to learn fully its nature and a method of control.

---

[1] Butler, E. J. Some Diseases of Palms. The Agricultural Journal of India, vol. 1, pt. 4, 1906, pp. 299–310. Reprinted in Bulletin of the Department of Agriculture of Jamaica, vol. 5, pts. 2 and 3, 1907, pp. 48–58.

[2] Stein, Pflanzer. Die Kokosnuss und deren Bearbeitung in Deutsch-Ostafrika. Der Tropenpflanzer, vol. 9, 1905, pp. 195–201.

[3] See "Coconut Leaf Disease in Ceylon and Portuguese West Africa," in Tropical Agriculturist, vol. 23, no. 7, January, 1904, p. 477.

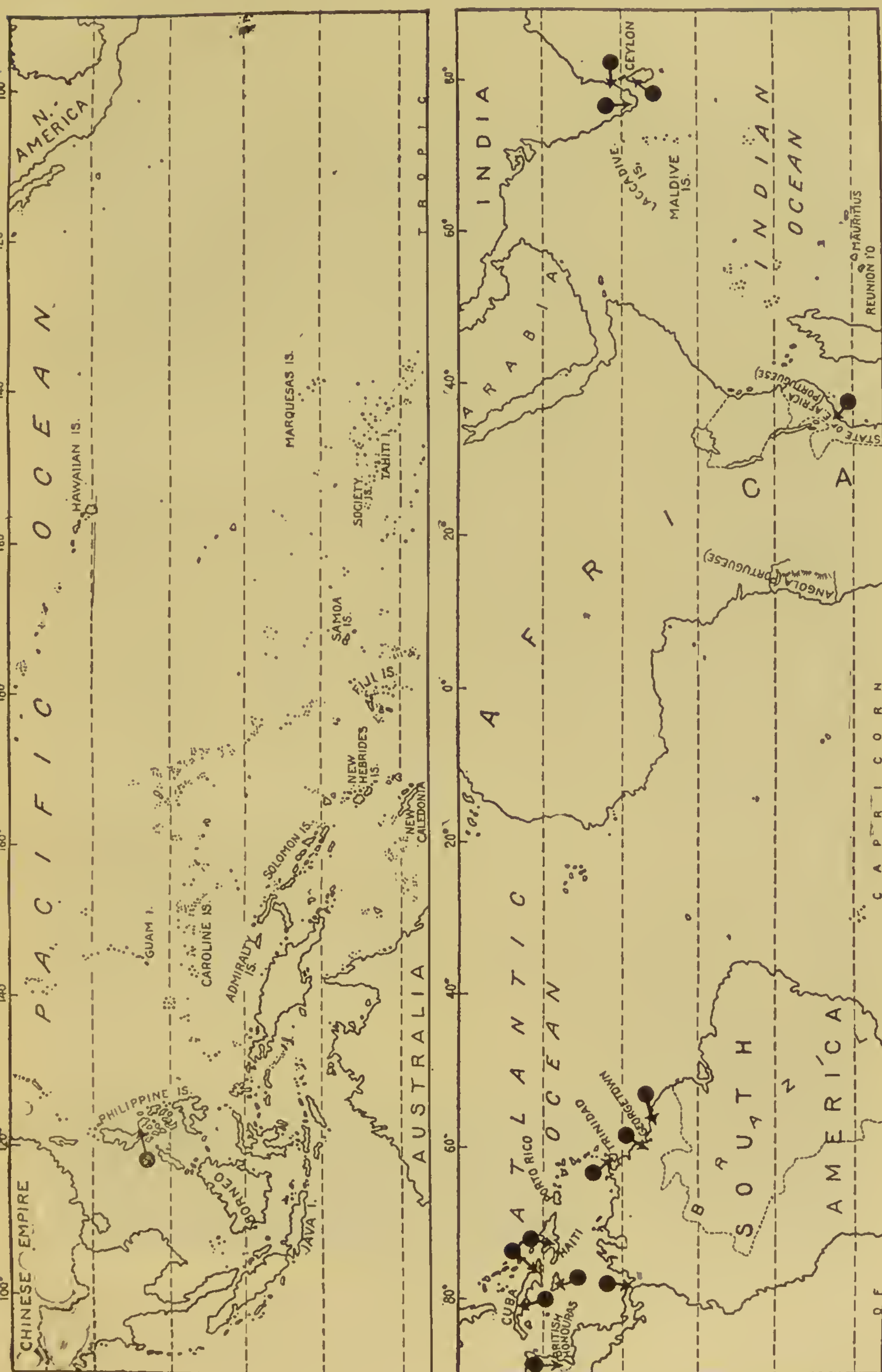


FIG. 5.—Map of the tropical countries of the world. The heavy arrows indicate the location of the bud-rot according to reports.

GENERAL INVESTIGATION OF THE DISEASE IN THE WEST INDIES.

Various investigators of the West Indies and of the United States have devoted some time to this disease of the coconut and have tried many different methods of controlling it, so that it is desirable to state here the results of their work.

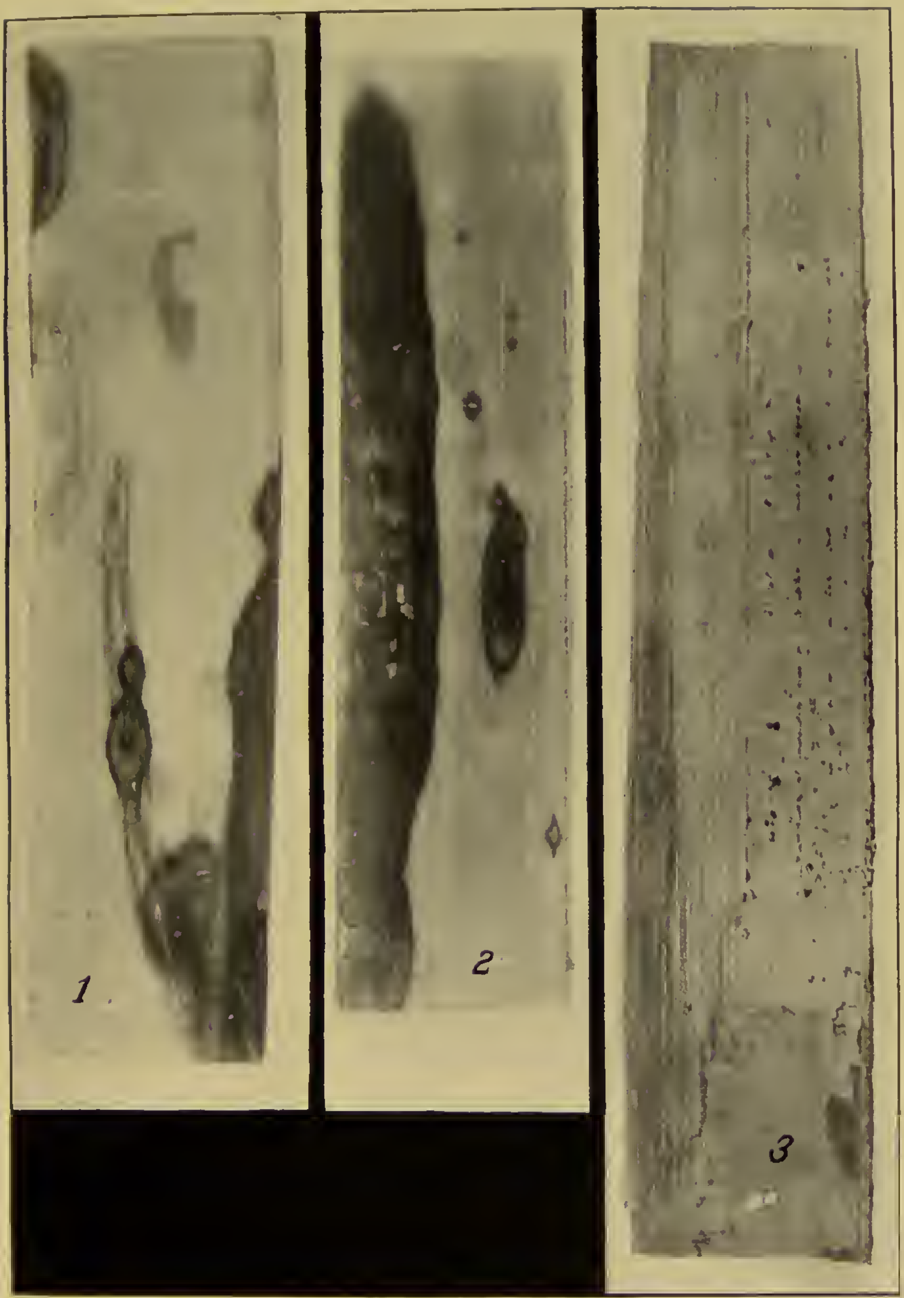
In 1901 requests from the coconut planters of Baracoa, Cuba, to the United States Department of Agriculture resulted in the assignment of Mr. August Busck, of the Bureau of Entomology, to investigate the disease. Mr. Busck started in August and traveled for several months over various districts. He investigated the purely entomological aspects of the disease and reported that while numerous insects were present in the rotting tops of the trees none of them seemed to occur in sufficient numbers to be considered responsible for the trouble. He was unable to find the palm weevil at all. Considerable fungous growth which was thought to be the cause of the decay was noted in the crown. The fungus was identified as *Pestalozzia palmarum* Cke., the cause of a widespread coconut-leaf disease.

In 1904 Dr. Erwin F. Smith, plant pathologist of the United States Department of Agriculture, continued the investigations. Most of his examinations were made in April. Before visiting the island he assumed, from Mr. Busck's statement that the terminal bud of the tree was involved in a soft rot, that the trouble was probably due to bacteria. Dr. Smith's own investigations, covering a period of about six weeks in eastern Cuba, confirmed him in this idea. Microscopic studies and numerous poured plates were made from trees in various localities—Baracoa, Mata, and Yumuri. He found only bacteria in the advancing margin of the diseased parts in the crown of the tree. Plenty of fungi and insects were present in adjacent rotted tissues, but these were considered to be of secondary importance. No inoculation experiments were carried on to prove the bacterial origin of the disease. Dr. Smith, however, retained interest in the subject and induced the writer to undertake this research.

Dr. Carlos de la Torre, of the University of Havana, in an address [1] at the university, admitted that the putrid condition in the crown of the coconut tree was due to bacterial fermentation, but claimed it should be considered as a consequence of the dying of the tissues and not as a cause. To him it was clear that the scale insects were the primary cause of the diseased condition. Unfortunately, he made no experiments to support his theories.

The Cuban Central Agronomical Station has also carried on work in the past two or three years to ascertain the cause of and a remedy

[1] Torre, Carlos de la. La Enfermadad de los Cocoteros. Revista de la Facultad de Letras y Ciencias, Universidad de la Habana, vol. 2, May, 1906, pp. 269-281.



FIGS. 1 AND 2.—BACTERIAL AND FUNGOUS SPOTS ON MIDDLE LEAVES OF COCONUT PALM. FIG. 3.—FUNGOUS SPOTS ON MIDDLE LEAVES.

for this disease. Mr. William T. Horne, until recently chief of the Department of Vegetable Pathology, has published a summary [1] of his investigations at the experiment station at Santiago de las Vegas and at Baracoa. His chief work has consisted of a search for a remedy for the disease, or means of controlling it. Search for a remedy has been prosecuted to some extent both in Cuba and in Jamaica, and the considerations of this aspect of the case will be discussed later.

As early as 1891 Mr. W. Fawcett reported a serious disease of the coconuts at Montego Bay, near the western end of the island of Jamaica. Since that time he and one of the agricultural instructors, Mr. W. Cradwick, have carried on investigations of this disease, giving more of their attention, however, to the study of methods of treatment than to ascertaining its cause. They have reported the disease from so many districts that it may be desirable to quote from their reports and correspondence.

Yesterday I inspected the coconuts at the railway station at Montego Bay. I find that the trees are dying there from rotting of the terminal bud in the same way that they are at Blue Hole and other places. * * * From my observations of yesterday I feel sure that the work is commenced by scale insects and the rot communicated by them. It does not start on the young flower sheaths, but on the old undeveloped ones; from these the rot spreads to the young flower sheaths, from these to the heart or terminal bud. (Dated Nov. 25, 1902. Unsigned, but kept in the files of the botanical department at Hope Garden.)

I cut down coconut trees at each place (Hopewell, Hanover, Sandy Bay, and Jericho) and fully succeeded in convincing the small settlers that my theory regarding the dying of the trees was the correct one. * * * I strongly advised them to cut down and burn any trees which were already in such a state as to render recovery impossible.

On Thursday, the 29th, I also visited Try-All estate, and with Mr. Brown cut down a coconut tree. This was a young tree apparently about 12 years old which had not long commenced to bear, growing on a hillside about 200 feet above sea level. It looked quite healthy, except that the nuts were dropping, but when we cut down the tree we found the rot had just reached the leaf bud and the youngest leaves were rotting.

The disease is evidently spreading. Trees are dying from Hopewell village to Green Island, but chiefly from Hopewell to Lucea. Some trees at Hopewell village were among the finest I have ever seen—about 7 or 8 years old, with the largest stems I have ever seen—just commencing to fruit, yet they were dying, one by one, from the rot of the heart leaf.

Trees are also dying at Barbican and Mosquito Cove, but I had not time to examine those closely.

At Sandy Bay and Jericho they are also dying.

There is quite a grove of young trees near Ramble, the property of Mr. Hudson. These are comparatively young ones, and are, I am afraid, doomed unless something can be done for them. (Dated Dec. 4, 1902. Unsigned, but in the files of the botanical department at Hope Garden.)

---

[1] Horne, W. T. The Bud Rot and Some Other Coconut Troubles in Cuba. Bulletin 15, Estación Central Agronómica de Cuba, July, 1908.

I entirely agree with Mr. Doull that the disease is spreading and no one, so far as I am able to ascertain, has tried the other remedies you suggested.

The disease is steadily thinning the coconut trees in and around the town, and its progress appears more rapid in the dry weather than in the rainy seasons. (J. W. Gruber. Dated May 4, 1892, addressed to W. Fawcett and on file at the botanical department at Hope Garden.)

In addition to the investigation of the disease by the staff of the botanical department of Jamaica, Prof. F. S. Earle, while on the staff of the New York Botanical Gardens, made studies of various maladies in Jamaica, in 1902, and among others investigated the coconut disease. His descriptions of it correspond exactly to the descriptions of the Cuban bud-rot. He came to the conclusion that it is a bacterial disease without, however, carrying on any infection experiments to prove this. He reports it as occurring not only in the extreme western part of the island, but also as far east as Port Antonio. He makes the noteworthy statement that at the time of his visit the disease was attracting little attention.

Mr. W. A. Murrill, also of the New York Botanical Gardens, visited Jamaica in 1908 and reported on the occurrence of the bud-rot in that island as follows:[1]

December 17 I left [Port Antonio] * * * and drove eastward along the north shore by Blue Hole and Priestmans River, and some distance beyond turned inland toward the John Crow Mountains until the road became impassable for vehicles, the trail continuing to Manchioneal. * * * Mr. Henslow pointed out trees 10 years of age that had been sprayed with Bordeaux mixture for the bacterial disease of the bud which has wrought such havoc with the cocoanut in Cuba, the Bahamas,[2] and elsewhere. The treatment has undoubtedly yielded good results, but the application of the mixture is sometimes a difficult problem.

The earliest published note of the occurrence of any serious coconut disease in Trinidad appears to be a letter from Mr. W. Greig to the imperial commissioner of agriculture for the West Indies, written June 30, 1905. Mr. Greig called the attention of the commissioner of agriculture to the fact that this disease was on the increase and that, according to the observations of Mr. August Busck, the disease in Trinidad was the same as the one studied by him in Cuba.

In September, 1905, Mr. J. H. Hart, formerly superintendent of the Botanical Gardens, made a personal investigation of La Retraite estate at Cedros. Here he found trees diseased from the ground upward, the stem showing a ring of red discoloration lying between the woody exterior and the softer interior. The discoloration became more prominent toward the growing point and appeared particularly at the base of the leafstalks and at the base of the embryonic spathes

[1] Murrill, W. A. Collecting Fungi in Jamaica. Journal, New York Botanical Garden, vol. 10, February, 1909, p. 25.

[2] Mr. Murrill's statement as to the occurrence of the bud-rot in the Bahamas can not be verified. It certainly is not present to any great extent on New Providence.

inclosing the floral organs. These all eventually became putrid, the leaves fell, and the tree finally died. Great quantities of bacteria, as well as fungi, were found in the affected tissues. Mr. Hart did not commit himself as to the cause of the trouble, but forwarded some of the material to the Imperial Department at Barbados, whence it was sent to the Department of Agriculture at Washington. Here the writer had the opportunity of examining it, and he is able to corroborate Mr. Hart's statement that the growing point was full of bacteria. From the particular specimens of Mr. Hart's material which are now preserved in the Laboratory of Plant Pathology at Washington microtome sections have been made and these demonstrate clearly numerous bacteria in the tissues and no signs whatever of a fungus.

During the latter part of July and the first of August, 1906, Mr. F. A. Stockdale, then mycologist of the Imperial Department of Agriculture, visited Trinidad and investigated the coconut diseases over the entire island. He reported on the same district that Mr. Hart investigated the preceding year, but contrary to Mr. Hart he found that the greatest number of diseased trees were injured primarily by a fungus rather than by bacteria. He investigated two maladies which completely destroyed the palms, one of which he called the "root disease" and the other the "bud-rot." He described the root disease as one in which the trunk shows a red discoloration toward the outside for a considerable portion of its length, while the decayed roots and the petioles are infected with a fungus which he considered as belonging to the genus Botryodiplodium. Eventually, when the vitality of the tree has been reduced, the terminal bud becomes involved in a soft rot, and the putrid mass then falls over and the tree dies. In describing the bud-rot, Stockdale says the roots appeared to be healthy and the stems showed no signs of discoloration, but the bud was involved in a vile sort of bacterial rot and eventually fell over. In the advancing margin of the rot usually there were only bacteria, but in a few cases there was some fungous mycelium. Mr. Stockdale concluded that the root disease was due to a fungus and the bud-rot to bacteria. In no case, however, did he make any infection experiments to prove the correctness of his theories. According to his descriptions, the tree suffering from the root disease differs from that affected by the bud-rot only in having a discolored trunk, diseased roots, and affected petioles, the rotted bud being common to both cases.

Mr. O. W. Barrett, in 1907, reported that of the diseased trees of the island about 95 per cent were affected with the root disease reported by Mr. Stockdale and only a very few cases were affected by bud-rot. Unfortunately no notes are given as to the appearance of

the diseased trees, so that Mr. Barrett's conception of these maladies is uncertain.

Dr. A. Fredholm presented before the agricultural society an article published in March, 1909.[1] He described a serious disease in which the trunk was normal and the roots usually so, while the terminal bud became disintegrated into a sour-smelling, whitish, semifluid mass, which, when examined under the microscope, was seen to be swarming with bacteria. The adjacent tissues, out to the petiole bases, were traversed by fungous mycelium which Dr. Fredholm believed to be the forerunner of the bacterial rot. He states that he considers Stockdale's root disease and the foregoing disease distinct, chiefly for the reason that he has never found the decay of the roots and the discolored stems present in the affected trees which he examined. He further states that he found a few cases of what was supposedly bud-rot, i. e., a putrid terminal bud full of bacteria and entirely lacking fungi. To substantiate his statements Dr. Fredholm obtained successful fungous infections (small spots), but he made no bacterial inoculations.

Mr. J. B. Rorer, formerly of the Laboratory of Plant Pathology, United States Department of Agriculture, has been mycologist of the Trinidad department of agriculture since early in 1909. Along with his other work Mr. Rorer has devoted some time to the coconut diseases and has given much attention to clearing out and destroying all diseased trees without waiting to ascertain the cause of the trouble. He is, however, investigating the nature of the various coconut diseases on the island, and writes the author as follows in a letter of June 6, 1910:

> So far I have cut down nearly 10,000 trees all told. There is no question as to the fact that bud-rot is present here, and my main object is to keep it from spreading, as I think it much more contagious than the other diseases. It has killed many trees at Toco and Laventille and is scattered all about the southern part of the island, but there is no doubt that from Iron Forest to Cedros the root disease has done much more damage—whether bud-rot helps it out is another question. * * * From what I have seen at Cedros the root disease seems to be distinct, and the trees may die from it, even if the bud is not affected. The roots are well rotted before the tree shows much sign of disease. One of the main points to be determined, it seems to me, is whether or not the true bud-rot organism is present in the rotting buds of root-sick trees.

As early as 1875 and 1876 Hon. William Russell reported to the Kew Gardens, England, that considerable damage was being done to the coconut trees in British Guiana. Outside of a few notes in local newspapers there have been few other reports of this disease until recently. In 1906 material was sent from Georgetown to Barbados to be examined by the mycologist of the Imperial Department of Agri-

[1] Fredholm, A. Diplodia Disease of the Coconut Palm (Society paper 367). Proceedings of the Agricultural Society of Trinidad and Tobago, vol. 9, pt. 3, March, 1909, pp. 159–172.

FIG. 1.—DISEASED COCONUT TREES 3 MILES INLAND FROM BARACOA, CUBA. FIG. 2.—TOP OF COCONUT TREE BLOWN OVER ON ACCOUNT OF ROTTED BASE OF THE CROWN.

culture. He reported that the tree was probably diseased by the bud-rot, without, however, making any detailed experiments to prove the etiology of this disease.

Besides the work done by the above stations some investigations of the coconut troubles have been made by planters in various districts, but no descriptions of such observations have been published.

INVESTIGATIONS OF THE DISEASE BY THE WRITER.

In January, 1907, the writer made a trip to the West Indies to continue the investigation of the coconut bud-rot begun by Dr. Smith. In order to obtain a thorough knowledge of the conditions under which the disease occurs and to ascertain whether it is the same malady in all the coconut districts, most of the important regions were visited and studied with care. In 1908 the investigations were continued in Cuba throughout almost the entire year, and again in 1909 and 1910 visits were made to the same island.

In Cuba the coconut industry is limited almost entirely to the Baracoa district, at the eastern end of the island (fig. 1). Here the stretch of land from Moa, upon the coast west of Baracoa, to Yumuri, on the east, is devoted largely to this crop. Coconuts are raised, not only on the coast, but also 1 or 2 leagues inland, where they are often interspersed with other crops.

From Moa to the River La Lisa there is at present no sign of the disease. The trees here appear healthy, although it is reported that some 20 years previous considerable of this trouble was experienced. In fact, one of the estates from which only 8,000 nuts per month are now gathered is said to have produced 70,000 per month in former times. Between the River La Lisa and the River Duaba the bud-rot has caused considerable havoc; cases are common along the shore plain, and also on the hills at an altitude of 60 meters or more. On the west shore of the Duaba it is widespread, but on the east shore and eastward to the outskirts of Baracoa, a distance of 1½ leagues, cases are rare. Coconut groves to the west of Duaba, shoreward, are in a neglected condition, and farther inland trees are thickly interspersed with bananas, cacao, and taro. In contrast to this coconut groves to the east are under clean cultivation and are not interspersed with other crops. A few cases appear in this clean district, as would be expected when it is so close to an infected area. The manager of these estates says that in 1906 forty trees appeared diseased, but by treatment he cured them all, though three cases recently reappeared. In a large grove immediately on the west shore of the harbor of Baracoa, on an estate called Jaiticito, there were in 1908, according to the owner, some 60 or more incipient cases, i. e., merely the dropping of the nuts without the destruction of the crown, but these were

cured. However, investigation of the grove in August, 1909, and again in 1910, revealed the presence of many more diseased trees.

The adjacent land to the south and southwest of the harbor of Baracoa is at the mouth of the River Macanagigua, and within a few years past this land was completely covered with groves in excellent condition. It is now a scene of the greatest desolation, many trunks standing without their crowns, and many with only a few leaves remaining upright. Following this valley inland the same scene of destruction is found. The slopes and the summits of the hills immediately between this valley and that of the River Duaba are covered with dead or dying coconut trees. In the Duaba Valley, at a point about 9 kilometers inland, there appeared during the year 1907 a number of cases of the disease which is now making great progress in the destruction of these excellent groves.

Turning back to the harbor of Baracoa one will see groves in devastation, not only on the south shore, but also on the eastern side of the valley. Here are several hundred cases where there were perhaps only two dozen two years ago. To the east of the town of Baracoa two large groves have been completely destroyed. A small grove of about 400 trees, having only a dozen cases two years ago, is now practically worthless, all of the trees being infected if not destroyed. Still farther east, along the River Miel, the same scene of destruction presents itself, there being a hundred or more of the bare trunks still standing and very few trees with green crowns.

The inland road from Baracoa east to Jamal runs 1 to 1½ leagues from the coast. It is well bordered by coconut groves which appear to be flourishing and show no signs of the bud-rot. From Jamal toward the coast the disease occurs in a few trees among many good ones. In a plantation on the hillside at Guirito many of the trees are dead or dying. From this town on toward the coast there are still many good trees, but at Mata Bay nearly all the trees are dead, and hundreds of headless tree trunks are standing. Many with the yellow tops yet remain, but only a few have green crowns and are bearing nuts. This description applies particularly to the south and east of Mata Bay. On the highlands just above Mata, at Guandao, a coconut grove, which formerly produced 12,000 nuts a month, now produces only 3,000. The trees that still remain are all bordering the shore, those that were inland having been destroyed. This estate has been replanted, and so far the young trees are doing well.

From Guandao to Yumuri, by the shore road, many dead or dying coconut trees appear, and the industry is at present of little importance. At the Yumuri River the land rises abruptly to a height of 200 to 250 meters to a broad table-land. About Yumuri, formerly a good coconut region, there is now little evidence of any coconuts

ever having been grown. One league in toward Sabana and half a league to the east a few dead trees and a very few live ones appear. This region is a thriving district, bananas, coffee, and cacao being successfully grown. Formerly coconuts were grown here. The extreme eastern end of the high mesa is largely a waste land and extends down to the seashore, stretching out $1\frac{1}{2}$ leagues to the extremity of the island at Maisi. No crops whatever are grown on this plain. The coconut growing of importance really stops at Mata.

On the coast road between Mata and Boma the coconuts appear to be thriving. One continuous grove extends over the table-land at an altitude of 75 to 100 meters with almost no sign of the disease. Coconut groves here are notably well kept, the underbrush being cleared away and no other crops interspersed. The Bay of Boma, which is midway between Mata and Baracoa, is bordered by coconut trees free from the disease. The trees between Boma and the River Miel, just on the outskirts of Baracoa, were free from the disease until the present year, some cases of its occurrence there now being reported.

After making a preliminary survey of the disease in Cuba the writer crossed over to Jamaica, traveling all over the island to ascertain the extent of the injury done by the bud-rot and to compare its symptoms with those of the Cuban disease. From the eastern end of Jamaica along the north shore westward and down to the southwest corner is an almost continuous stretch of coconut groves. They are not by any means confined to the seacoast, but flourish inland 6 leagues from the sea at an altitude of 600 or more meters. From Kingston by rail to Anotto Bay coconut groves appeared at intervals, but in no case did there seem to be any serious disease. From Anotto Bay along the coast to Port Antonio many coconuts may be seen, some with bare trunks and stumps, but no appearance of bud-rot. These stumps are said to be the result of a destructive hurricane in 1903. Several specimens of diseased trees were examined at Port Antonio. Two of these trees had the center of the crown entirely missing, and merely a fringe consisting of the lower leaves remained; these leaves, however, were still in their horizontal fresh green condition. The manager of the estate stated that the cause of this trouble was lightning, but there was really no evidence that such was the case. The very center of the crown was in a dry-rotted condition, suggesting the work of insects. Many of the trees on one part of this plantation were seriously injured by the scale insects, and on another part, on a steep hillside, they appeared to be suffering from lack of water, judging from the extreme yellowing of the leaves. In none of these cases did there appear to be any suggestion of the bud-rot.

According to the report of the planters about Port Antonio, there was no indication of the presence of bud-rot at the extreme eastern end of Jamaica, so that no inspection of these groves was made at this time by the writer. A recent report (p. 24) indicates its presence in this district, although apparently in a mild form. No cases were apparent between Port Antonio and Annotto Bay. Between Annotto Bay and the Parish of St. James no disease has been reported, but in the Parishes of St. James, Hanover, and Westmoreland, the three extreme western parishes of the island, there is abundance of the bud-rot. On the road from Montpelier to Savanna la Mar, a few trees (at Petersfield and Amity Cross) have the typical appearance of this disease. Occasional cases appear at distant intervals from Savanna la Mar along the coast to Green Isle, and on to Lucea, also still farther to Montego Bay, and, according to report, a few kilometers to the northeast of Montego Bay. The disease was said to be very bad at Negril Point. In most of these places, however, the diseased trees had been cut out and destroyed. In one grove just to the south of Montego Bay there were a number of typical cases. Some of these trees were cut down for the writer and showed in every way the symptoms of the Cuban disease.

The conditions of culture in Jamaica are, as a rule, very good, in great contrast to the conditions in Cuba. The underbrush is kept out, the fallen leaves and other débris are burned up, and the planting of other crops between the trees is little practiced. Bananas are often kept running until the coconuts come into full bearing, when they are cut out. Thus, in every way the Jamaican planter has much better conditions under which to combat the disease. It is reported that in past years the bud-rot has done considerable damage. It is certain, however, that the disease is now well under control. It occurs chiefly along a stretch of about 20 leagues of the coast line and for the most part in isolated cases, probably not over 50 cases existing at the time of this investigation. In one grove at Negril Point, as the result of the neglect of the trees, the disease was allowed to progress. This was the only seriously affected grove in the district. *In Jamaica, then, the bud-rot has been put under control by keeping the diseased trees cut down.* Whether or not some conditions might arise favorable to the rapid spread of the infection from a single tree it is impossible to say. It is believed that the few planters in Jamaica who have the disease in their coconut groves do not cut down on an average more than one-tenth of 1 per cent of their trees annually. It is a question whether the value of these trees could begin to pay for any treatment of them.

In April, 1907, the writer visited all of the coconut-growing districts of Trinidad. The industry in this island is very extensive

DISEASED COCONUT TREES AT BARACOA, CUBA.

almost the entire coast line on every side being given up to coconut trees. The important places are the great strip of land along the eastern coast from Manzanilla to Mayaro, and to Galcota, and the entire Cedros Point with the adjacent shores. Bud-rot appears to have caused great havoc in at least two places. At Laventille, a league or so east of Port of Spain and bordering on the Caroni River, great devastation appeared. The accompanying diagram (fig. 6)

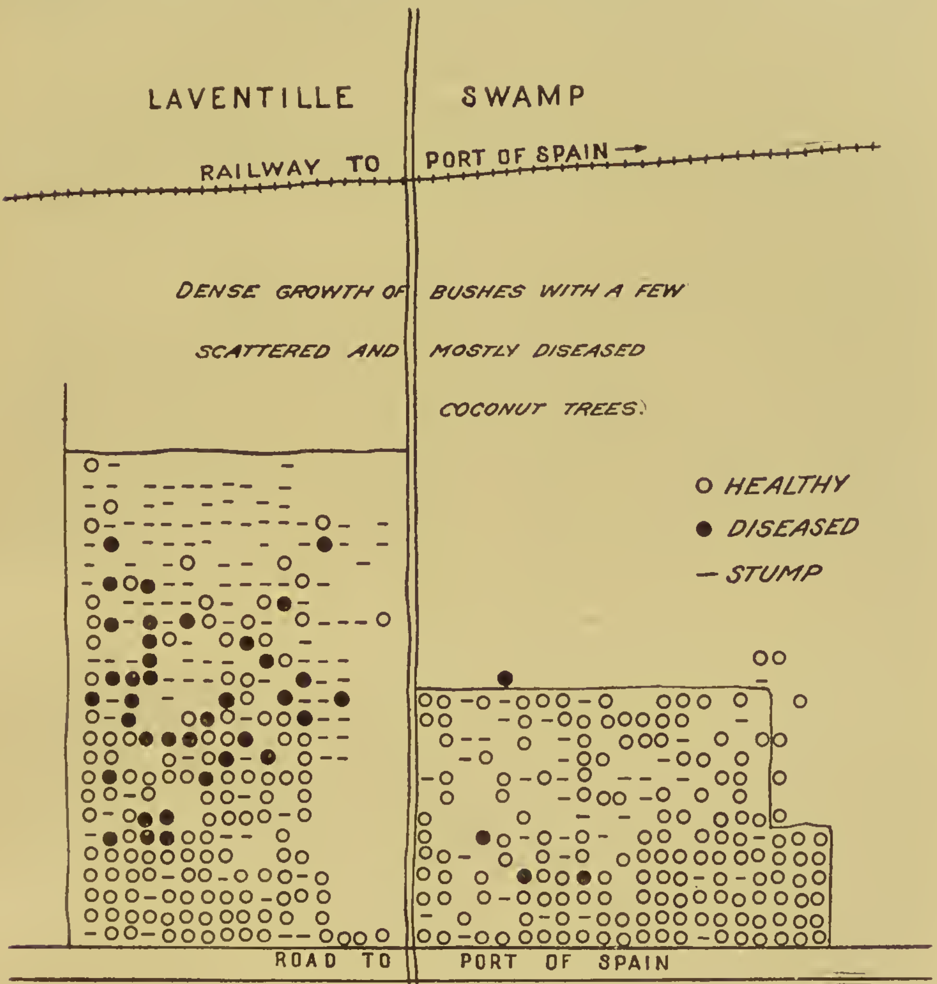


FIG. 6.—Diagram showing diseased coconut trees in Trinidad.

illustrates the condition of one estate at this time. As is here shown, a large area lying between the present growth of coconut trees and the railroad track was occupied by a dense growth of bushes with an occasional, and usually diseased, coconut tree. This was all a part of one estate, and formerly the entire area illustrated was covered with coconut trees in good condition. The place rivaled some of the worst in Cuba in the number of dead and dying trees. The attorney for the estate estimated that about 85 per cent of the trees had been

destroyed. On a large part of the plantation the disease had reduced the trees to short stumps; on other parts to tall trunks with no tops, or with tops brown and hanging down, or consisting of two or three or half a dozen leaves. Healthy green trees were few in number. A public road separates this estate from one to the north on which there were many coconut trees, scarcely any of which were affected. The diseased area is a very low, swampy, and poorly drained place which was overgrown with underbrush and had been entirely left to itself as to the spread of the disease and general cultivation. Water is reached at a depth of 45 centimeters and often less in the wet season. The soil under the surface is a heavy, sticky clay. The diseased trees had all the appearance of bud-rot, the lower leaves having turned brown and for the most part fallen off, only a few leaves remaining more or less upright. Some trees were cut down and examined as to the exact nature of the affected parts of the crown. The rot in the tissues was found to correspond exactly to that present in diseased trees in Cuba and in Jamaica. All of the unhealthy trees of this estate, with the exception of one or two in which very clearly the cause was insects and in which the soft rot was absent, presented the typical appearance of the trees infected with bud-rot. The unfavorable conditions under which these palms were grown, i. e., in wet, clayey soil, together with the fact that the estate had been entirely neglected for many years, undoubtedly had some influence on the spread of the disease. Mr. Stockdale, in his report, considered the majority of the trees of this estate to be affected primarily with what he calls the root disease, and Mr. O. W. Barrett, in his investigations in 1907, confirmed Mr. Stockdale's opinion, but this is contrary to the observations of the writer. It is sufficient to say here that the disease called the "root disease" is very little understood and has no proved cause. According to Mr. Stockdale, who describes it, very frequently the crown of such a diseased tree becomes later involved in a soft rot, leading one to think, in many cases at least, that the root trouble is secondary and that the bud-rot or crown disease is the primary one. Careful observations leave no doubt that practically all of the diseased trees in this district are affected in their crown with a soft rot, the symptoms of which are typical of the well-known bud-rot. This statement does not at all oppose the idea that the trunks or roots of some of the trees so diseased may contain some fungus, or other organism quite different from that which occurs in the crown, but not the cause of the bud-rot.

Another district similar to Laventille is at Point d'Or, near La Brea and the Pitch Lake. This estate had not been in cultivation for a number of years. Consequently it was heavily overgrown and

the malady had obtained considerable headway. During the year 1906 the manager had undertaken to clear up and eradicate the disease. After a year's work he was of the opinion that as fast as infested trees were cut down and their tops burned new cases appeared, and he seemed to have the prospect before him of the entire grove going to destruction. In contrast to the swampy condition of the Laventille estate, this place is hilly and well drained and apparently suitable for coconuts. Close examination of these trees, by cutting down and opening the crown, disclosed exactly the same condition as that of the trees affected by bud-rot in eastern Cuba.

From Point d'Or southwest coconut groves appeared to be in good condition. At Guapo there had been a few diseased trees that resembled those with the bud-rot. As a matter of fact, the trunks of these trees within 3 feet of the ground showed a red discoloration, and in all probability this is what Mr. Stockdale refers to as the root disease. Only three cases were observed here, so the prospect did not appear to be very serious.

From Guapo southward to the end of Cedros Point coconuts appeared to be in good condition, although it was reported (1905) that there were many diseased trees at Cedros Point. On Mr. Greig's estate of some 110,000 trees, there appeared to be only a very few affected ones, and on examination of the trunks these few showed the red discoloration characteristic of the root disease as described by Mr. Stockdale. Mr. Hart investigated the disease in 1905 on a part of Mr. Greig's estate, and reported the presence in the crown of the soft rot swarming with bacteria. The fact that Mr. Greig kept his estate in excellent condition, i. e., all of the diseased trees cut down and destroyed and the fallen leaves and other débris picked up, probably accounts for the presence of so few cases. The question as to whether the bud-rot or the root disease is the primary trouble on this estate needs further investigation.

It is reported [1] that in the Siparia district and along the swamp lands below Princestown there has been a great loss of coconut palms, and the description of the disease certainly answers very well for the bud-rot. On the east coast of the island, along which for almost the entire length is a narrow strip of coconuts, there appeared to be absolutely no sign of any serious trouble, the trees presenting a most healthy appearance and bearing well.

From these reports it will be seen that while the bud-rot has been extremely destructive in certain parts of Trinidad, it must be noted that these parts have in general been greatly neglected or else lie in low, swampy situations, such as are entirely unsuitable to coconut growing.

[1] Stockdale, F. A. Coconut Palm Disease. Trinidad Royal Gazette, Feb. 14, 1907, pp. 361-362.

After investigating the diseased trees of Trinidad the writer went to Demerara, British Guiana, and there, with the help of the former superintendent of the Botanical Gardens, Mr. A. H. Bartlett, carried on studies of the disease in that region. Coconut palms occur scattered along the coast. They are found chiefly on the islands in the mouth of the Essequibo River and on the adjacent mainland, and in the southern part of Demerara, near Mahaica and Mahaicony. The islands of the Essequibo River are merely sand drifts which have been overgrown with vegetation. These have been partly cleared and on them coconut palms are grown. A disease has been reported from this locality, and from a personal examination of the trees externally the writer is inclined to think that it is the bud-rot; but the cases are comparatively few. At various intervals along the railway from the Essequibo to Georgetown and from Georgetown southeast to Mahaicony, which is largely a coconut district, there appeared isolated cases of what seemed to be the same disease. This entire coast line is at the level or below the level of the sea at high tide, so that sea defenses are built and canals are maintained with pumping stations for proper irrigation and drainage of the land. Under such circumstances the meadows are for the most part wet and partly under water. Much of the land is too wet for the coconut palm. A considerable number of diseased trees, some of which could be definitely said to have bud-rot, were found at Mahaicony. On one estate on which the soil was rather heavy and poorly drained certain trees were selected for examination. They were the only affected ones on the estate. All of them showed the typical conditions—the central leaf bud dead or dry, and low down in the crown a typical soft rot. Insects were present, to be sure, as is usually the case, but in no such numbers as to connect them directly with the disease.

The coconut industry of British Guiana is still on a rather small scale, owing chiefly to the fact that the land is more suitable and more valuable for sugar planting. In 1877 the exports amounted to 1,500,000 nuts, while now they are only 500,000 per year. The presence of this disease, though not very virulent in form, probably discourages more planting of coconuts under conditions which are also otherwise unfavorable for their growth, i. e., a heavy soil together with an excessive quantity of water present.

The island of Porto Rico as one of our possessions has been of great interest to us, especially as no disease of the coconuts appears to be present. The coconut industry of the island, although far below the value of that of sugar cane and tobacco, is of considerable importance and is by all means worthy of protection and extension. The fact that the bud-rot disease is so prevalent in nearly all the coconut-growing regions of tropical America, in regions not far

removed from Porto Rico and in districts from which seed coconuts are sometimes brought into this island, made it eminently desirable to prove that this district was free from this disease, and when this was proved, to ask for legislative control of importation of seed coconuts into the island.[1] The fact that a former botanist of the agricultural station in Porto Rico and a former agent in charge of the station both expressed the belief that the disease was present, led the writer to examine carefully all of the groves on the island. In investigations which the writer made in 1907 around almost the entire coast and along the railroads no cases were found. The only part not visited at that time was between Ponce and Humacao. More recently, in December, 1910, an examination has been made of the groves between Ponce and Guayama and again between San Juan and Barceloneta, but no cases of bud-rot were found.

In 1910 the writer saw five or six trees on the coast between Anasco and Corsica which had very much the general appearance of bud-rot. Closer examination in the present year (1911) showed the diseased tissues to be somewhat similar to that of bud-rot but not typical. Further studies have been made by Mr. G. L. Fawcett, of the Mayaguez Experiment Station, but the presence of true bud-rot has not as yet been demonstrated.

A number of the trees in various places appeared to be in an unhealthy condition; leaves were yellowing or broken, or the lower ones had fallen, but in no case did it appear like bud-rot. In the groves along the north and west sides of the island at frequent intervals trees were found from which lower leaves had fallen, but the remaining fronds were green and to all appearances healthy, and in many cases nuts were still produced. A number of trees were also found whose crowns had rotted off entirely. These trees were always isolated cases and did not resemble those affected by bud-rot, but rather suggested insect work.

Coconut groves extend at intervals all along the coast (fig. 7). An almost continuous strip of them extends eastward from San Juan to Loisa and beyond to Luquillo. Thence beyond Cape San Juan down the coast to Naguabo the groves are very few. From Naguabo to Humacao another extensive grove extends along the beach. From Humacao westward to Ponce coconuts are reported to be infrequent. From Ponce westward small groves appear occasionally until the west coast is reached, where an extensive and almost continuous grove extends from the southwest and northward along the coast to Mayaguez, and on northward to Aguadilla. This west

[1] In 1907, Dr. Erwin F. Smith drew up a bill for the Porto Rico Island Legislature, looking to the prevention of the introduction of this disease, but in the form in which it finally passed the law is of no value for the protection of the island. Fortunately the legislature of 1910–11 passed a bill which covers the ground quite satisfactorily.

coast has by far the most coconut trees of any part of the island. From Aguadilla along the north shore to San Juan these palms appear at intervals in small groves, but never in large numbers. Trees of these groves as far as examined had no sign of bud-rot, nor any serious infectious disease.

The examination of the coconut industry in Porto Rico concluded the preliminary survey of the writer in regard to the distribution of bud-rot. Visits have also been made to the few groves on New Providence Island in the Bahamas, and to small groves scattered along the coast of Colombia and Venezuela; but in none of these districts was the coconut industry of any great importance, nor was any very serious disease found among them. The writer has not visited southern Florida, but understands from Prof. P. H. Rolfs and Dr. E. A. Bessey that none of the groves in that region have as yet shown any signs of this disease.

FIG. 7.—Map of Porto Rico. The location of coconut groves is indicated by dots.

STRUCTURE OF THE COCONUT TREE.

In order to understand the nature of the experimental work on the cause and methods of control of bud-rot it is necessary to know thoroughly the structure and arrangement of the parts of the coconut tree. The tree consists of a single unbranched trunk crowned by a huge rosette of leaves. Each of these leaves at maturity may be anywhere from 4 to 7 meters in length and from 1 to 1.5 meters in width. The leaf consists of a single heavy rachis bearing the simple pinnæ. This rachis, or leafstalk, broadens out at the base so as to form a complete sheath about the trunk. (See fig. 8, petiole *a* broadening out into a leaf sheath.) From its thin, fibrous character the sheath is commonly called the strainer. This forms a tough, tight binding about the inclosed portions. An average mature tree has from 25 to 30 leaves. The distance from the lower leaves of the under part of the crown to the center of the crown, the base of the highest

and youngest leaf, is from 1 to 1.5 meters (fig. 9, the distance between points x and y). As leaves appear in the center of the crown they are upright and tightly folded, like a closed fan, gradually opening and assuming a more oblique and later a horizontal position as they mature. The great length of the leaves gives them the appearance of considerable flexibility as they wave in the breeze, but it is impossible to bend away the central leaves and get down anywhere near the center, this fact being due partly, of course, to the leaf sheath, and also partly to the rigidity of the stalk. At the inside base of every leaf is a flower bud which enlarges and splits open, allowing an elongate sword or spathe to develop

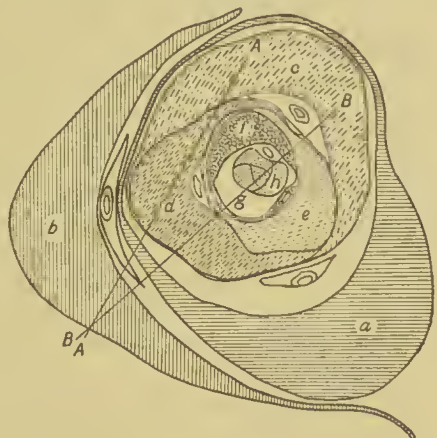


FIG. 8.—Diagrammatic cross section of bud of the coconut palm inclosed by some of the outer leaf sheaths: *a, b, c, d, e, f, g, h*, Successive petioles, each extending laterally into a leaf sheath. Immediately adjacent to each leafstalk is a sword.

FIG. 9.—Diagrammatic longitudinal section of bud of the coconut palm, including the top of the crown: *C*, Trunk; *A*, heart; *S*, sword; *SC*, sword sheath; *LS*, leafstalk; *R*, rachis or fruitstalk.

to a length of 1 to 1.3 meters (fig. 9). Then the sword itself splits longitudinally and allows the flower spike to open out Each spike bears both pistillate and staminate flowers. An average tree will have perhaps 10 spikes of nuts and a dozen or so nuts on a spike. Usually 30 or 40 nuts are set from a flower spike, but seldom more than 10 to 20 mature. The arrangement of the crown of leaves in rosette fashion furnishes an excellent receptacle for rain, which runs down and soaks into the fibrous sheath and serves to keep the tender growing part in a constantly moist condition. The base of the leaves also serves as a catchall for fallen flowers, mature nuts, and the like—forms of débris which tend to rot close to the trunk. Under normal conditions there is no injury, but under certain conditions the débris and the constant moisture held in the strainer furnish a means by which the disease may pass from an innocuous condition among the hard tissues of the outer leaves to that of a most virulent pest in the inner delicate growing tissues.

The trunk of the coconut tree is an almost uniform mass of fibers and fundamental tissue from the roots to the crown, and is a hard, woody material. The roots are very numerous and radiate horizontally in all directions from the tree, extending practically as far as the leaves of the crown. The roots are almost uniform in size, about 1 centimeter in diameter.

The accompanying figures 8 and 9 are intended to show the relation of the different parts in cross section and in longitudinal section. It is difficult, or impossible, to show all parts in their proportionate sizes, but their relative position is more important, and it is believed that this may be clearly seen. The cross section is such as would appear if made through the line BB of the longitudinal section after the removal of the external leaves.

FIELD STUDIES OF THE DISEASE.

INFECTION STUDIES.

To determine the infectiousness of the disease was the first problem. That bud-rot was communicable from tree to tree was accepted by some, but ignored or disbelieved by others. By many it has been thought due to something in the soil or to the climatic conditions, and various applications have been made to the base of the tree in the hope of curing it. Insects eating the roots and working in the trunk or in the crown have also been considered as causes. It has likewise been claimed that a mechanical injury, such as a bullet piercing the tender heart tissues, would produce a rot of the crown. It is safe to say that most of the reasons given as to the causes were based on inaccurate or incomplete observations, together with a lack of any experiments to substantiate them. The rapid spread of the disease in itself seems good evidence of its infectious nature, for it does not stop in one valley or one grove, but frequently spreads over a hillside and into the next valley, always beginning in a small way and from that spreading sporadically over the entire grove. If the disease were due to soil or to climatic changes, many or all of the trees would show signs of the rot about the same time. It could hardly be supposed that this might be accounted for by variation in individual resistance, since in the end most or all of the trees contract the disease.

Assuming the trouble to be infectious, it has been a mooted question as to whether fungi or bacteria were the cause of it. Prior to 1887 Dr. Ramos, of Havana, maintained that a fungus (*Uredo coccivoro*) was the cause. This has been upheld by many, but by others—notably bacteriologists—it has been disputed a priori on the ground that fungi seldom cause a putrid fermentation such as is to be found in the crown of the diseased coconut tree, while

bacteria frequently do, and from the observations of Dr. Smith it has been seen that only bacteria are in the advancing margin of the decaying tissues. Moreover, it has since been claimed that *Uredo coccivoro* is nothing else than the normal scales of the coconut leaf.[1]

Dr. Davalos, of the bacteriological laboratory of Havana, isolated in 1886 what he claimed to be *Bacillus amylobacter*, which he believed was the cause of the soft rot.[2]

Dr. Plaxton, of Jamaica, in 1891, before the Institute of Jamaica,[3] showed under the microscope some slides of a micrococcus which he thought was probably the cause of the coconut disease. In other parts of this paper the writer has quoted many investigators ascribing the cause of the disease either to bacteria or to fungi. The opinions as to the cause of the disease are so various, and hence, reasons for methods of treatment so unsatisfactory, that it has seemed eminently desirable to carry out a clear series of experiments to settle, first, the infectiousness of the disease; second, if infectious, whether due to fungi or to bacteria; and third, if possible, to isolate the organism causing the disease.

BACTERIAL INOCULATIONS.

Owing to the height of the trunk and the great size of the crown, inoculation of coconut trees is difficult. The rot is peculiarly one of soft tissues, so that in order to be effective the bacteria must be placed in the interior among these soft tissues. From the bottom of the crown to the growing point there is commonly a distance of 1 to 1.5 meters (fig. 9, from x to y), so that the exact location of a spot suitable for inoculation is hard to determine. Inoculations made below the heart into the trunk fail to produce the rot, since these tissues naturally soon harden as a part of the mature tree. If, on the other hand, the inoculation be made above the heart amid the growing leaves, their extremely rapid elongation takes the inoculation point out from the surrounding soft tissues. The inoculated tissues then become green and membranous and thus resist the advance of the rot. The point of easy inoculation is less than 0.5 meter above or below the growing point, and rather near the center of the tree. (See fig. 8. Inoculation on line *BB* is desirable; inoculation on line *AA* would seldom be successful.) This often requires an inoculating

---

[1] Tamayo, Dr. La Epifitia de los Cocoteros. Revista de Agricultura (Cuba), vol. 9, 1889, pp. 557–8, 570–1, 584–5.

Torre, Carlos de la. La Enfermadad de los Cocoteros. Revista do la Facultad de Letras y Ciencias, Universidad de la Habana, vol. 2, no. 3, May, 1906, p. 274.

[2] Davalos, Dr. Revista de Agricultura. Boletín Oficial del Círculo de Hacendados do la Isla do Cuba, vol. 9, no. 29, 1889.

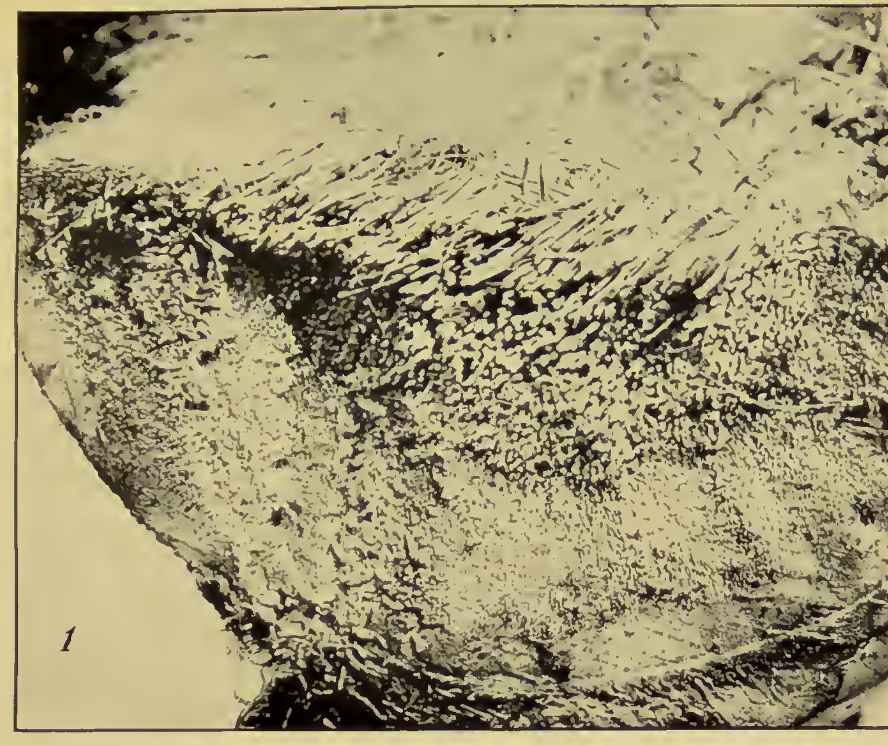
[3] Plaxton, Dr. Journal of the Institute of Jamaica, vol. 1, 1891–1893, pp. 43–44.

instrument that will reach at least 30 centimeters from the surface of the trunk inward. These conditions probably account for the difficulty which different investigators have had in obtaining infections. Naturally, the right organism to cause the disease must first be obtained, although infection can probably be accomplished by using the juice of a seriously diseased tree. The matter of isolating specific bacteria is of course desirable, but presents greater difficulties. Ever since beginning the work the writer has occasionally obtained excellent rots at the point of infection with apparently different organisms. It has been said that any mechanical wound of the heart tissue will cause it to rot and die. This statement is proved to be untrue by the check inoculations and by some inoculations into the heart which failed to take and produced no rot whatever.

During the investigations of the writer in various parts of the West Indies, from January to June, 1907, diseased material was obtained from many different trees affected with the bud-rot, and bacterial organisms were isolated from these tissues. The cultures isolated consisted, in general, of two types: One which produced, usually, round, wet shining, white, and semiopaque colonies with raised surfaces; and one (the type most abundant) which produced colonies of very thin growth, spreading rapidly over the plate in an irregular fashion, often sending out long radiating branches. This most abundant type was also white, wet shining, and semitransparent. Comparisons were made of the cultures obtained from Cuba, Jamaica, Trinidad, and Demerara, but they were not found to be identical in their cultural characteristics. Notwithstanding this, several of the cultures were taken the following February, 1908, to Baracoa, Cuba, and there inoculated into apparently healthy trees. Together with these cultural inoculations wounds were made with a sterile instrument to serve as checks. Table I gives the data concerning all of these inoculation results.

TABLE I.—*Inoculations of coconut trees, February and March, 1908.*

Source of culture.	Character of colony or culture.	Inoculation. No.	Date of inoculation.	Date of examination.	Results.
Jamaica	Thin, irregular, white.	151	Mar. 10	Mar. 16	Decided rot; diseased part cut off and the tree left.
Demerara	....do	430	Feb. 21	Mar. 9	No result; inoculation too low.
Cuba	....do	392	Feb. 24	Mar. 10	Too low; still a small distinct rot.
	....do	417	Mar. 10	Mar. 16	No external symptoms.
	....do	189	...do	...do	Decided rot; tree left standing.
	....do	150	...do	...do	Do.
	Raised, round	421	...do	...do	No result; tree left standing.
Demerara	Thin, irregular, white.	385	...do	...do	Do.
	No notes	151	Feb. 24	Mar. 10	No infection; inoculation too low.
	....do	337	...do	...do	No result.
	....do	419	...do	Mar. 9	No result; too low.
	....do	417	...do	Mar. 10	No external symptoms.



BACTERIAL INOCULATION, SHOWING DESTRUCTION OF FUNDAMENTAL TISSUE ABOUT WOODY FIBERS OF COCONUT PALM. FIG. 1.—CROSS SECTION. FIG. 2.—LONGITUDINAL SECTION.

TABLE I.—*Inoculations of coconut trees, February and March, 1908*—Continued.

Source of culture.	Character of colony or culture.	Inoculation No.	Date of inoculation.	Date of examination.	Results.
Cuba:					
164......	Juice of diseased tissues.	78	Mar. 1	Mar. 16	No result; inoculation too low.
201......	.....do............	408	Mar. 10	...do....	No external symptom of disease.
201......	Juice of diseased tissues poured on, not injected.	434	...do....	...do....	Do.
	Check tree inoculated with sterile agar.	156	Feb. 24	Mar. 10	Absolutely no effect toward rotting the tissues.
	Check; inoculation hole but nothing injected.	292	...do....	...do....	Do.

It will be seen from a study of the table that the inoculations were scarcely successful in proving the bud-rot to be due to bacteria. It will be remembered, however, that these were the first inoculations made by the writer, and apparently most of them were made in parts of the tree seldom affected by the bud-rot. This was owing to the difficulty of locating the precise area suitable for inoculation, a feature that has since been overcome by rather extended studies. All that can be said for this series of inoculations is that some of them, at least, showed a rot typical of the bud-rot. In view of the fact that the check inoculations did not affect the tissues in this way at all, it would seem to indicate that those few cases which had any rot or decay were actually caused by the organisms inoculated, notwithstanding the fact that in other cases certain organisms injected did not cause such a rot. No material was obtained from these artificially diseased tissues in order to reisolate the organisms injected.

In the summer following this series two more inoculations were made: On June 22 tree 173 was inoculated with a bacterial culture. Externally it showed no signs of infection until October 21. The tree was then cut down and carefully examined, when it was found that the entire heart of the tree was in a soft, putrid condition, typical of the bud-rot. Previous to inoculation this tree was bearing poorly, but not showing any distinctive signs of the disease.

Tree No. 380 was inoculated on July 22 as follows: All traces of the lower leaves were cleared away, so as to expose as much as possible of the white tissue about the bases of the remaining leaves; then small pyramidal pieces, 2 centimeters deep, were cut out, infected agar was put inside, and the pieces were replaced. Several of these inoculations were made, and bandages of wet cotton were tied about the wounds in order to maintain a constantly moist condition. On August 6 this tree was examined and none of the inoculations appeared to have taken effect. Either the right organism

was not used or the tissue at the bases of the leaves was too hard to be easily infected.

The following November a new series of inoculations was made in the same grove, near Baracoa, Cuba. Cultures were obtained from four different trees, and inoculations were made into 13 others. Just as in previous isolations of organisms from diseased material, two types of colonies had predominated—the round and the irregular, both of them white—so in the isolations from this series these two types predominated and both of them were used for the inoculations. The results are shown in Table II.

TABLE II.—*Inoçulations of coconut trees, November 2 and 3, 1908.*

Source of culture. Tree No.	Character of colony.	Inoculation into tree No.	Date of inoculation.	Date of examination.	Results.
96	Thin, round, white	337	Nov. 2	Nov. 10	Good rot about inoculation hole.
	Round, convex, white.	380	...do.....	Nov. 13	Good soft, white rot.
155	Thin, irregular, white.	417	...do.....	Nov. 10	Slight rot.
	Thin, round, white....	421	...do.....	Nov. 11	Water-soaked, discolored area about inoculation hole and soft rot in tissues below heart.
	Thin, irregular, white.	422	...do.....	...do.....	Good soft, white rot about inoculation hole.
	....do..............	423	...do.....	Nov. 13	Do.
296	Flat, round, white....	248	...do.....	Nov. 17	Extensive soft, white rot.
	Thin, irregular, white.	52	Nov. 3	Nov. 20	Fairly good, soft, white rot.
373	....do..............	78	...do.....	Nov. 17	Excellent soft, white rot, very conspicuous.
	....do..............	64	...do.....	Nov. 19	Inoculation high and one side of center; some rot in midrib of leaf.
	Round, convex, white.	153	...do.....	...do.....	No decided rot, but distortion of tissues just below heart.
	Thin, irregular, white.	189	...do.....	...do.....	Excellent soft, white rot.
	Convex, round, white.	150	...do.....	...do.....	Do.

Table II, in contrast to Table I, shows that many of these inoculations were successful. Full descriptions omitted from the table are here given:

No. 337 was examined 8 days after inoculation, when it showed rot just below the heart (Pl. VIII) and along the inoculation hole and adjacent tissues. The central leaves of this tree also had a wet rot considerably higher than the inoculation, but there was no visible connection between the two.

No. 380 was examined 11 days after inoculation and showed a soft, rotted area about 2.5 centimeters in diameter, running the entire length of the inoculation hole. This rot was the typical soft, white rot. The strainer was browned and rotted over a large area downward from the inoculation hole (Pl. IX, fig. 1).

No. 417 was examined after 8 days and showed only a slight rot, a water-soaked area about 3 centimeters in diameter, which extended from the inoculation hole; but it did not show the typical soft, white decay of the tissues. The middle leaves were also diseased higher up and apparently independent of the inoculation.

No. 421 was examined after 9 days, and showed an area about the hole very distinctly water soaked and discolored. There was clearly a soft rot in the tissues below the heart not definitely connected with the inoculation.

No. 422 was examined after 9 days and the inoculation hole was found to be directly through the heart and to have caused a good soft, white rot. The effect on the tissues was not limited to the softer ones in the interior, but was also evident on the harder tissues of the strainers and leaf bases.

No. 423 was examined after 11 days. The inoculation was found to pass immediately below the heart and to have caused an excellent soft, white rot, which affected a considerable area of the tissues. There were also on this tree numbers of the leaf-base spots.

No. 248 was examined after 15 days, and the inoculation was found to have passed 15 centimeters above the heart and a little to one side. The excellent soft, white rot was, however, spreading rapidly on all sides and above as high as 30 centimeters from the inoculation. The extreme upper parts of the leaves were perfectly healthy. In places where the inoculation passed through the strainers and leafstalks the rot extended in areas anywhere from 1 to 30 centimeters in length; all of the tissues within 20 centimeters of the heart were badly rotted (Pl. IX, fig. 2).

No. 64 was examined after 16 days, and the inoculation was found to be rather high and to one side of the center. A very little rot was present in the midrib of the outer leaf. All the other tissues were not affected.

No. 153 was examined after 16 days; very little sign of any rot was present. The inoculation had caused a distortion of the inner tissues, but no other noteworthy change.

No. 189 was examined after 16 days and showed a splendid typical soft rot just below the heart. The affected area extended for a distance of 10 centimeters above and below the inoculation.

No. 150 was examined after 16 days and showed an excellent inoculation which had passed but 5 centimeters below the heart; the rot from it had passed into the heart itself. Toward the outer side of the inoculation it passed through a young sword which, as a result, had become rotted and blackened at the tip on the inner tissues.

It thus appears that the successful infections were brought about apparently by a variety of organisms. Following the examination of these inoculations, material therefrom was carefully selected and sterilized on the outside surfaces so as to permit the transfer of uncontaminated portions into bouillon tubes, for the purpose of pouring plates and thus isolating the organisms which were present in the rotted tissues. Many plates were made and a variety of organisms were isolated. In general two types seemed to be predominant; one type, most conspicuous, was the round white colony with a raised surface, wet shining, and semiopaque. These colonies in the course of two or three days attained, on a +15 nutrient agar, a diameter of 4 to 8 millimeters, but seldom became much larger. The other abundant type was of thin growth whose colonies spread rapidly over the plate, i. e., where in one day's growth they might have a diameter of 2 centimeters; in two days the growth might be 5 or 6 centimeters.

Series of experiments were made in the Laboratory of Plant Pathology at Washington, D. C., for the purpose of comparing the cultures from the different trees. Plates of nutrient agar poured from the diseased material showed in general the same type of colony as was

formed by the organism used in the inoculation. Comparison of the various cultures showed about half to be alike in their reaction in litmus milk. Several of the organisms were inoculated into various vegetables, and in a few cases good rots were obtained in cucumbers.

From this work it seemed most probable that the organism causing the bud-rot was the one which formed the thin, very much branched type of colony. Cultures of it were taken to Cuba in August, 1909, and inoculated into various trees. As will be seen in Table III, none of these cultures had any effect whatever in rotting the trees.

At this time other isolations were made from naturally diseased trees, and inoculations were made with these as indicated in Table III.

TABLE III.—*Inoculations of coconut trees, August 9 to 14, 1909.*

Source of culture.		Character of colony.	Inoculation into tree No.	Date of inoculation.	Date of examination.	Results.
Tree No.	Date.					
339	Nov., 1908	Good, irregular, white growth.	442	Aug. 9	Aug. 25	No rot.
			444	...do.....	Aug. 23	Do.
			406	Aug. 10	...do.....	Do.
			447	...do.....	...do.....	Do.
			331	...do.....	Aug. 25	Do.
209	Aug. 7, 1909	Irregular, thin	505	Aug. 12	...do.....	Extensive soft, white rot.
		Irregular, thin, white	502	...do.....	...do.....	Decided soft, white rot, but not extending much beyond inoculation hole.
		....do	508	...do.....	Aug. 24	Splendid rot, soft and white, along inoculation hole.
		....do	507	...do.....	...do.....	Fair rot in inner sword.
		Round, convex, white.	501	...do.....	Aug. 25	Extensive typical soft, white rot.
252	....do	Irregular, thin, white.	504	...do.....	Aug. 26	Good soft, white rot.
		....do	506	Aug. 4	Aug. 24	Splendid rot along inoculation hole.
		....do	503	[1]	....	

[1] Not inoculated.

Table III shows that various successful infections were again made, and also that at this time, as in the spring of 1908, the inoculations were made with a variety of organisms. In more detail than is given in the table the results of these inoculations are as follows:

No. 505 was examined 13 days after the inoculation and showed an excellent soft, white rot extending a distance of 60 centimeters above the inoculation hole.

No. 502 was examined after 13 days, when it showed a very soft, white rot about the inoculation hole, but it was not very extensive.

No. 508 was examined after 12 days, when it showed a splendid soft, white rot. The lower part of the inoculation hole was entirely reduced to a thick white liquid. One of the inner swords was blackened at the tip in the interior. The inoculation passed 3 to 4 centimeters above the heart tissues, in the best possible place, and took effect. The resulting rot extended about 45 centimeters above the hole and also down into the heart tissues. The middle leaves of this tree some distance above the inoculation were in a perfectly healthy condition.

No. 507 was examined after 12 days, when it showed a fair rot in one of the inner swords.

FIG. 1.—BACTERIAL INOCULATION OF COCONUT PALM No. 380, SHOWING DISCOLORATION OF THE SHEATH.

FIG. 2.—BACTERIAL INOCULATION OF COCONUT PALM No. 248, SHOWING DECAY OF INNER TISSUES.

No. 501 was examined after 13 days; it showed an excellent decay for a distance of 60 centimeters above the inoculation and a considerable distance below it.

No. 504 was examined after 14 days, when it showed a good soft rot extending 4 centimeters about the inoculation hole.

Three other inoculated trees, Nos. 601, 602, and 603, were not examined.

Following the examination of these inoculated trees, plates were poured from the diseased tissue, and an attempt was made to isolate the organism present in the diseased parts. By comparing the predominant organism isolated in each case from these trees with the organism inoculated into the trees, it was found in a good many cases that several of these organisms seemed to be identical. This fact lent encouragement to the use of some of these isolated organisms for reinoculation into another series of trees. Unfortunately, it was not possible at this time to carry on the work in Cuba, so that inoculations were made into seedling coconuts in the greenhouse at Washington. These coconuts were not by any means desirable for this purpose, being decidedly stunted in their growth, and in consequence their tissues appeared to be drier and more woody than is natural to the tree. It was rather to be expected that greater difficulty would be encountered in producing the rot in these seedling coconuts than if the inoculations were made in good healthy trees. Table IV gives the results of these inoculations.

TABLE IV.—*Greenhouse inoculations of coconut trees, September 24 to 29, 1909.*

Source of culture. Tree No.	Character of colony.	Inoculated into tree No.	Date of inoculation.	Date of of examination.	Results.
503 (N series only)	Thin, white, irregular.	503 *a*	Sept 24	Oct. 27	A small, rotted area and a large water-soaked area.
506	do	506 *a*	Sept 29	do	Water-soaked area for a distance of 1 centimeter from inoculation hole.
505	do	505 *a*	do	do	Excellent brown, water-soaked condition.
508	do	508 *a*	Sept. 24	Oct. 16	Rot extended about 7.5 centimeters above hole and 2.5 centimeters below it in inner tissues; typical soft, white rot in inner tissues.

The results of the inoculations, shown in Table IV, are not nearly so striking as in the case of those made into the trees in Cuba. As has been brought out in the previous discussions, the inoculations can be made to take only where the disease is found to occur naturally—in the heart tissues—and the heart tissues of all of these sprouted coconuts were very limited. In each of these inoculations the tissues were well water-soaked, which certainly was due to the presence of the bacteria, as the check inoculation failed to show any effect whatever. One inoculation especially—that of 508—seemed

a good one, extending for 7.5 centimeters above the inoculation hole and 3 centimeters below it. As these inoculations were made with a small injecting needle, the wound caused by the instrument itself was very slight.

So many successful inoculations with bacterial cultures inevitably lead one to the conclusion that a rot in the heart tissues of the coconut palm identical in every respect with the bud-rot is caused by these bacterial organisms. That such a condition is caused by mechanical injuries such as those of the inoculating instrument is disproved by the check inoculations which produced absolutely no rot at all. It has been suggested that the disease was carried by means of the inoculating instrument to the inner tissues from affected outer tissues. The possibility of this can not be denied. It is impossible to obtain evidence of the fact that the bases of the leaves and swords toward the interior are certainly free from disease. From the general appearance of the tree one may judge all its parts to be free or infected, but this is the best that can be done. However, this objection does not lie against the hothouse experiments in Washington, because the nuts were obtained from a disease-free district.

While this uncertainty may affect the probability of these inoculations causing the rot in individual trees, yet in view of the fact that the same organism as that injected into the tree has been isolated from the artificially diseased tissues, the probability seems greatly in favor of this particular organism, or else it suggests strongly that if any bacteria were already present in the tissues and caused the infection, they were of the same kind as those injected. Now that these reisolated organisms have been inoculated into other trees and have induced typical soft rots, from which the same organisms have been reisolated, proof seems complete that at least a certain kind of bacteria, namely the kind used in the successful inoculations just described, does cause the diseased condition of the coconut palm known as bud-rot.

No experiments have been carried on to prove that this is the only organism causing the bud-rot. The fact that cultures of apparently different organisms did produce decayed tissues certainly suggests that other organisms than the one isolated may produce the same effect. At the same time slight differences in the appearance of colonies on agar can not be regarded as specific. The question of the power of other organisms to produce the same appearance is an interesting one and undoubtedly will arise again with further work. It seems sufficient for the present (1) to have proved that this condition is due to a bacterial infection and (2) to have isolated a particular organism which is capable of producing the disease.

FUNGOUS INOCULATIONS.

It has been supposed by some people that Pestalozzia and Diplodia or Botryodiplodium play an important part in producing the disease, since one or more forms of these organisms are found in abundance on the central rotted leaves. Very frequently brown spots occur on the middle of young leaves of trees which are apparently free from bud-rot, i. e., which show an entire absence of a putrid condition of the heart. These brown spots range from minute ones to those 5 centimeters in diameter, and they seldom become larger, but remain in a dry condition, presenting the same appearance in the older and mature leaves. Such spots are, without much doubt, caused by both Pestalozzia and Diplodia, both of which form tiny black pustules in the center of a diseased area. If, as has been said, the spots remain dry, they seldom cause any serious damage to the leaves. On the contrary, if bacteria also are present, causing a wet, slimy condition, it is a beginning of the bud-rot. The bacteria destroy the leaf tissues immediately under the epidermis, leaving an extremely thin, paperlike covering over the destroyed parenchyma cells and the firm woody cells of the leaf veins. This condition occurs frequently in an infected region. There may be but a half-meter of diseased tissue, consisting of both a luxuriant, black, sooty covering of the fungus and the slimy bacterial growth. This tissue may be 1 to 1½ meters below the top of the central leaves and as far above the heart tissues. The slimy condition progresses downward into the more fleshy tissues, where it becomes a typical soft rot. The advancing margin of this rot almost never contains fungous filaments but swarms with bacteria and forms the typical bud-rot. The slimy condition extends upward only so far as it may have fairly soft tissue for food material, and is protected by surrounding leaves which keep it constantly moist. Higher up in the crown where the leaves begin to unfold the tissues are harder and more membranous and are exposed to the wind and sunlight which furnish conditions unfavorable to the growth of the bacteria. The fungous infection seldom extends to the top of the diseased leaves, which turn brown and dry and supply a poor substratum for the fungous growth. Under the foregoing conditions, when both fungi and bacteria were present in incipient cases, it was a puzzling question as to which was the cause of the diseased condition. In a number of trees, however, the middle leaves were affected with the fungous spots alone, and, as previously mentioned, it may be seen that either fungi or bacteria may be the first present. It is probable that germination and growth take place best in the presence of unusually moist conditions among the tightly packed middle leaves or on some of the frequent droppings of the tree frogs, lizards, and various insects which are found present in such places.

The scale insects, as well as cockroaches, earwigs, ants, and other insects, may cause small mechanical injuries that give the fungus foothold. In order to determine whether the fungus was infectious small pieces of seriously diseased tissue were loosely bound with wet cotton over the slightly scratched surface of some leaves, quite low down, where the tissues were just turning green. After six days the trees were examined and the two leaves which had been so thoroughly wrapped as to remain moist were infected, while those which had dried out were not. On one of them a typical fungous infection extended 3 centimeters beyond the inoculation and on another for the distance of 8 centimeters beyond the wounds. The four other inoculations showed no growth, the inoculation material itself having dried out, which rendered infection impossible. While this was but an incomplete experiment, carried on in a small way, the flourishing condition of the fungus on the tissues of the two infected leaves would indicate that, given proper conditions of moisture on slightly diseased or wounded tissues, the fungus would make good growth. The fact, however, that these two successful cases did not advance further and did not develop into the slimy condition and progress downward into a soft rot tends to prove, if any proof is necessary, that the soft-rotted condition in bud-rot is not caused by the fungus.

SPREAD OF INFECTION.

For the spread of disease caused by a parasitic organism some carriers of the infection are essential. The bud-rot has been shown on foregoing pages to be due to a bacterial organism and in some of the pages that follow it is demonstrated to be due to a particular organism. The means of spreading the infection from tree to tree has, however, not been ascertained. It has been claimed by some that wind is the chief means and by others that insects play an important part.

The arguments in favor of wind are based largely upon the observations that the distribution of the infection is sometimes in the direction of the prevailing wind. This condition appears to be true in some cases, but unfortunately for the argument the cases are quite as common in which the spread is against the wind. Not only this, but the new infections are more sporadic than would be expected. The disease occurred in Baracoa Harbor, serious first on the south side, and from there spread to the east side, skipping over a group of several hundred trees. This spread was off to one side of the usual course of the wind. On the west side of Baracoa Harbor there is a large estate which covers both sides of a small hill, on the one hand facing the sea and the breezes and on the other hand away from the sea and in the direction of the wind. The disease has been on the windward side

for at least the last three years, but is only at present beginning to be serious on the leeward side.

Considering the examples as a whole, it seems difficult to find a definite case of the spread of the infection in any one direction. The most favorable example for this theory is that of the condition of the groves in the Macanagigua Valley and toward El Yunque—that is, to the south, nearly in the direction of the prevailing wind. The disease spread from the valley to the hilltop inland and then crossed the hilltop and has since gone a few miles farther south into the valleys. Even this is not a clear-cut example, as a closer study of it reveals. As already stated, a careful study was made of one grove in this region during the year 1908–9. Figure 10 shows the condition of the grove on March 10, May 28, August 5, and October 21, 1908. The lower right-hand side of each section of the diagram represents the windward side of the grove. Section *a* shows, however, that there are more healthy trees standing on that side than on the leeward side, represented on the diagram by the upper left-hand corner. The straight horizontal dash indicates diseased trees that have been cut down or destroyed. In sections *c* and *d* it will be seen that the leeward side is almost destitute of standing trees, either diseased or healthy. It will further be found that there are many trees represented in the lower left-hand corner that have become diseased and that are somewhat to the windward of the first lot. Perhaps the most striking thing shown by the diagram is the sporadic nature of the spread of the infection. It seems difficult to attribute this to wind.

An entirely different argument and one opposed to the idea of wind as the distributor of the germ is the nature of the infection. The diseased tissues consist of a soft, wet rot in the heart of the crown and surrounded by the hard tissues of the leaf bases and sheaths. It is difficult to conceive of the wind getting at such a location and blowing about moist bits of tissue. It is true that many forms of bacteria are carried about in the air, but they are forms that are able to withstand considerable desiccation and are usually, if not always, sporulating forms. The organism causing bud-rot is not, so far as known, a spore former and can not withstand sufficient drying to permit of its being blown about like so much dust.

In contrast to the idea of wind as a disseminator of the germs is the belief of many that insects, or birds, or some form of animal life is responsible for this trouble. If this were the case, the spread of the infection would occur in just such a sporadic manner as shown in figure 10, sections *a* to *d*. As seen in the field the evidence all tends to point in this direction. If the insects that may carry the disease are flying forms, it would explain the occasional apparent

spread of the disease in the direction of the wind. The kind of insect or animal life responsible, however, is not yet determined. A great variety of forms, as described on other pages, has been found in the tops of both diseased and healthy trees. In the infected

FIG. 10.—Diagrams showing the progress of the bud-rot in a coconut grove at Baracoa, Cuba, from March 10 to October 21, 1908. The four sections of the diagram indicate the condition of the grove on the dates specified.

tissues themselves are usually found in abundance larvæ of flies and earwigs. In some very deep portions, even where it was difficult to imagine the possibility of access to flies or other insects, numerous larvæ occur.

It seems most likely, in the face of all this, that insects are the carriers. In view of the fact that they are found so commonly on diseased tissue it seems very possible that they might carry off some organism either attached externally to their bodies or legs, or taken internally in the course of feeding and subsequently excreted upon healthy tissues. With the exception of fly larvæ, earwigs are the most common insects. Some of these were collected from diseased tissues, carefully washed for 20 seconds in mercuric chlorid (1:500), then rinsed in water and placed in tubes containing Dolt's medium (p. 79). The insects were well crushed in the tubes so that any bacteria from the digestive tract would come in contact with the medium.

Transfers were made in Washington from these tubes (eight in number) to beef bouillon, and an attempt was made to isolate the coconut organism. Plates poured from four out of the eight tubes showed among many a few colonies resembling the coconut organism. Transfers from each of the four plates to litmus milk gave the typical reaction. Transfers from the litmus milk to nitrate bouillon, to fermentation tubes with neutral red, to gelatin, and to Dunham's solution, likewise gave the reaction of the coconut organism. Thus, this investigation of the insects, while much too incomplete for proof, indicates that the disease-producing organism may be found in the intestines of earwigs; and such being the case, these insects may be at least partly responsible for the distribution of the disease.[1]

It seems possible also that turkey buzzards may be responsible for carrying the disease germ from tree to tree. These birds are found in all the tropical localities where the bud-rot occurs, and they may commonly be seen in diseased trees. That they feed on the infected tissues is uncertain, but it seems probable that they do. Such tissues have a very bad odor—at times it reminds the writer strongly of an abattoir—and it is likely that they are attracted by it.[2] It does not seem improbable that such birds may feed on the material or at least get some of the organisms on their feet, after which it is an easy matter to spread the infection. In the hope of ascertaining the probability of the coconut organism occurring in the digestive tract of these buzzards some of the fresh dropping was placed in tubes of Dolt's medium. Several plates were made, twice for each of the several bouillon tubes. In every case a small proportion of the resulting colonies appeared to be the coconut organ-

[1] In one of the last trees cut down at Yumuri, Dr. Smith found two larvæ in the rotting bud not far from the sound tissues. These larvæ were of a gray color and probably similar ones had been overlooked. They were put into a clean petri dish and brought to Washington. On the way they pupated and later one of them passed into the imago state and was identified by Mr. Coquillett as *Hermetia illudens* L., a common scavenger fly of the Tropics.

[2] Dr. Smith states that twice, at Baracoa, buzzards swooped down on the rotted hearts of palms he had laid aside for study and would have carried the material off if he had not made a frantic rush to protect it.

ism. From these colonies transfers were made to litmus milk, which in every case reddened the litmus and coagulated the milk and subsequently became partially bleached as in the true coconut organism. Transfers were made from these litmus-milk tubes to fermentation tubes containing peptone, dextrose, and neutral red, and in every case the typical greenish-yellow reaction took place in the closed arm of the tubes and gas was produced, usually the ordinary amount, though in one series of tubes it ran as high as 70 to 90 per cent. Transfers were also made from the litmus milk to nitrate bouillon, and tests after 48 hours' growth at 32° C. showed a reduction of the nitrate in every case. Similarly, transfers from the nitrate bouillon to beef gelatin +15 kept in the thermostat at 37° C. for 48 hours and afterwards placed in an ice box to harden, failed to show any liquefaction of the gelatin. Cultures of these organisms in Dunham's solution when tested for indol showed a good pink reaction.

Thus, in all these tests certain bacteria responded to the usual tests for the coconut organism. The proportion of this organism to the total amount found in the dejecta of the buzzard was, however, small.

In the tops of palms rats have frequently been found as well as other animal life, but it does not appear likely that these serve as carriers of the disease. An attempt was made to isolate the coconut organism from excreta of rats, but without success.

It will be seen in the following pages that the organism causing bud-rot is *Bacillus coli*, or at least an organism indistinguishable from it. This organism is one that has been known for a long time as being of almost universal distribution and one that is commonly found in the intestines of man and some of the lower animals. The widespread distribution of *Bacillus coli* would seem to coincide with the widespread occurrence of the bud-rot, for, as described on other pages, a disease identical with this appears to occur in almost all parts of the tropical world. If *Bacillus coli* is to be found in so many countries and is the cause of bud-rot, how is it that there are any regions free or apparently free from this disease? This is a question that naturally arises, and one that does not lend itself to an easy solution. To illustrate this: *Bacillus coli* is shown to be the cause of the coconut bud-rot in Cuba, but this disease does not appear to occur in Porto Rico. *Bacillus coli* undoubtedly occurs in Porto Rico but does not cause the coconut disease. Why is it that the organism that causes the disease in one locality does not cause it in another when it is present? A plausible explanation is in the supposed absence of the particular carrier of the infection in these

regions where the disease does not occur.[1] As there is no complete evidence for or against this, the question must for the present remain unsettled. An explanation might, however, be sought in considering what passes for *Bacillus coli* as a group of organisms, the members of which, while alike in the usual cultural characters, possess varying pathogenic properties.[2] The only other possible explanation is a difference in the soil or climate. The necessary evidence to support such a theory is, however, entirely lacking.

---

[1] An earwig similar to or identical with the Cuban species has been found by the writer in Porto Rico, but the turkey buzzard is either entirely absent or at least rare on that island.

[2] Since this statement was written Daniel D. Jackson has published a very instructive paper (Journal of Infectious Diseases, March, 1911, pp. 241-249) in which he maintains *Bacillus coli* to be a group of related species, divided by him as follows: *B. cummunior* (Durham), *B. communis* (Escherich), *B. aerogenes* (Escherich), *B. acidi-lactici* (Hueppe). The first two species are separated from the second two by their gas production with dulcit and the first of each of these two groups may be separated from the second by its gas production with saccharose.

Each of these species may be separated into four possible varieties in accordance with their gas production with mannit aud raffinose. Three varieties each of the first and fourth species are now known, two varieties of the third, and all four possible varieties of the second group have been found.

In a diagram 21 varieties of *Bacillus coli* are given, four of which are as yet unknown.

Bacillus communior (Durham).—Variety A_1: Fermentation with gas production with dextrose, lactose, dulcit, saccharose, mannit, and raffinose; milk coagulated, nitrate reduced, motile, and indol positive. Variety A_2: Fermentation the same as A_1; motile, reduces nitrate; differs from A_1 in not producing indol. Variety B: Ferments with gas production with dextrose, lactose, dulcit, saccharose, and mannit, but forms no gas with raffinose. Also distinguished by no coagulation in milk even after heating and by slow formation of gas in dulcit. This latter test usually takes three days for the gas formation to become active. Motile, indol positive, nitrate reduced. Variety C: Fermentation with gas production with dextrose, lactose, dulcit, saccharose, and raffinose; forms no gas with mannit; milk coagulated, nitrate reduced, motile, and indol positive.

Bacillus communis (Escherich).—Variety A: Fermentation with gas production with dextrose, lactose, dulcit, mannit, and raffinose; no gas formation with saccharose; motile, indol slight, nitrate reduced. Variety B: Fermentation with gas production with dextrose, lactose, dulcit, and mannit; no gas production with saccharose and raffinose; milk coagulated, nitrate reduced, motile, and indol positive. This appears to be the most common variety of *B. communis.* Variety C: Fermentation with gas production with dextrose, lactose, dulcit, and raffinose; no gas production with saccharose or mannit; nitrate reduced, indol positive, motile. Variety D: Fermentation with gas production with dextrose, lactose, and dulcit; no gas production with saccharose, mannit, or raffinose; nitrate reduced, indol positive.

Bacillus aerogenes (Escherich).—Variety A_1: Fermentation with production of gas with dextrose, lactose saccharose, mannit, and raffinose; no gas production with dulcit; indol positivo, nitrate reduced, motility negative; viscous growth on agar and in lactose bile; in the latter it can be drawn out into a long, thin string. Variety A_2: Fermentations the same as A_1; motile, indol negative, nitrate reduction positive; differs from A_1 in being less viscid or stringy when touched with the needle; in being motile, and indol negative. Variety A_3: Fermentations and all tests with one exception same as A_2; liquefies after 26 days; differs from A_2 in being slightly liquefying in gelatin stab after about 26 days. The total gas and percentage of CO_2 is high when grown in dextrose broth and particularly in liver broth. This species has been at times grouped with *B. cloacae* (Jordan), but the former never fails to produce gas with lactose, whilo typical *B. cloacae* apparently always gives negative results, when dextrose-free lactose solutions are used. Another marked distinction is that truo *B. cloacae* after rejuvenating is always strongly liquefying, while *B. aerogenes* A_3 never liquefies beforo 20 days, even after careful rejuvenation ovor long periods. Variety B_1: Forms gas with dextrose, lactose, saccharose, and mannit, but no gas with dulcit and raffinose; nonmotile, indol negative, nitrate reduced; viscous growth on agar and in lactose bile. May be drawn out into a thin string by using a platinum needle. Variety B_2: Differs from B_1 in being motile, indol positive, and nonviscous in lactose bile.

Bacillus acidi-lactici (Hueppe).—Variety A_1: Fermentation with gas production with dextrose, lactose, mannit, and raffinose; no gas production with dulcit and saccharose; nonmotile, indol positive, nitrate reduction positive. Variety A_2: Fermentation same as A_1; indol positive, nitrate reduction positive; differs from A_1 in being motile. Variety B: Fermentation with gas production with dextrose, lactose, and mannit; no gas production with dulcit, saccharose, or raffinose; milk coagulated, nitrate reduced, motile, and indol positive. Isolated by Melia in nine strains from human feces. Often exceeding in numbers all other varieties of bacteria in feces. Variety D: Gas production with dextrose and lactose; no gas production with dulcit, saccharose, mannit, or raffinose; indol positive, nitrate reduced.

While it appears probable from the studies of the writer that insects are the carriers of infection, it is still of the utmost importance that this matter be ascertained definitely.

REMEDIAL AND PREVENTIVE EXPERIMENTS.

In the early part of this work the writer had before him as the picture of bud-rot the conception most general among planters and investigators, that of a soft-rotted condition of the bud. Almost at a single glance one could state definitely that a tree in such a diseased condition had no hope of being cured either by indirect or direct methods. The single growing point of the tree being rotted, there was no power within the tree to produce another growing point.

In order to ascertain if the disease did not attack other tissues before getting into the heart or growing point, the writer repeatedly ascended many trees to the summit, and there made careful observations on the condition of the central leaves during the course of a year, as has been noted on other pages.

A month or so after the first examination it was observed in some cases that trees which had been free from rot in the center became badly diseased. It commonly happened in such trees that one or more flower spikes just opening revealed dark-brown wilted tips. Trees were also found with these discolored flower spikes and with healthy central leaves. This condition suggested the idea of removing the one diseased spike and watching further development. This was done, as shown in the following record:

Tree No. 96.

March 7, 1908: The tree had 5 spikes of nuts and 1 good sword visible from the ground.

May 28: Nine fairly good spikes and two good swords were found.

June 8: Same condition.

June 25: There were 9 spikes, bearing about 100 nuts, and 4 good swords. One spike with nuts just set showed dark-brown water-soaked tissue at its base—the only sign of disease in the tree. The middle leaves were healthy. The diseased spike and the adjacent tissues were cut out. Owing to the compactness of the leaf bases and their strainers at the base of the crown it was impossible to say that the infection may not have been carried to other parts. No satisfactory means of disinfecting the whole crown was at hand.

July 21: One of the green swords had become brown and dead. Six of the nut spikes were empty.

July 24: Of the 100 nuts on June 25 there remained about 40. Removed by pruning the dead sword and all of the spikes having no nuts, together with their subtending leaves. In all, cut off 19 leaves and 10 spikes. There were left 7 good leaves and 2 green swords. The central leaves were still healthy.

August 5: No change.

October 21: The two swords as well as the middle leaves were brown and dead. There was a soft rot in the heart tissues.

Similar conditions were noted and followed through their course of development in numerous other cases. Each time it was made reasonably certain by careful examination that there was no disease of the middle leaves, and then all of the lower infected leaves and spikes were removed, one by one, and the trees left standing. In the course of a few weeks the outermost flower spikes and swords which had been left in an apparently healthy green condition now became discolored and wilted. These were removed and only green ones left. This was repeated until finally in every case a typical rot appeared in the central leaves and quickly penetrated to the heart, thus killing the tree. In all, 21 trees were pruned through a series of months, as described above, and in each case the disease finally lodged in the central leaves. These experiments seem to furnish good evidence as to the origin and course of the infection. Although no inoculations have been made to prove it, yet in no other way can the condition be interpreted than that the water-soaked spots at the base of the leaves and the black wet-rot of the strainer is the precursor of the central bud-rot (Pl. X, figs. 1 and 2). Microscopic studies of the water-soaked spots, showing numerous bacteria, confirm this idea.

With this evidence, then, as to the course of the disease, the question as to remedies or preventives can be studied with a more thorough understanding of the conditions.

REMEDIES.

When it was found that the disease often first occurred at the inner leaf bases and gradually passed to the central leaves it was hoped that some application might be made to the crown that would destroy the incipient infections.

If the leaf-base spots and external strainer rot could be removed before the rot had penetrated to the deeper tissues there seemed some hope of success. The methods of accomplishing this have been varied. To remove the diseased tissues at the base of the leaves the writer resorted to pruning. As mentioned in previous paragraphs, 21 trees were treated in this way. It appeared impossible, however, to remove all of the infection. The germs could pass through the strainer, and they might have been present in the tissues without showing any sign of rot. For that reason it was difficult to tell how much to prune. Cutting off merely the diseased area did not seem efficient. As many as 15 to 20 leaves could be removed—leaving 5 or 6—without seriously injuring the tree. To carry this further would so weaken the crown that the first strong wind would blow it over entirely. In this work, as carried out by the writer, the trees

remained undiseased at the heart from one to three months, but eventually they all succumbed.

In contrast to the plan of removing infected parts, other experiments were carried out to counteract the progress of the disease. It was hoped that by applying certain chemicals the affected tissues would be poisoned, or perhaps cauterized. Planters in various regions have applied salt, iron sulphate, Bordeaux mixture, etc., to the crown, presumably with this idea in mind. The writer tried salt, copper sulphate, and Paris green, but here, as in spraying to destroy the insects, it was found impossible to reach all of the infected portion. The experiment with these chemicals progressed as follows:

Tree No. 240.

February 26: It bore 9 spikes of nuts and 2 good swords. Appearanee healthy.

March 11: Same.

May 28: Same.

June 22: There were a dozen fallen rotted nuts.

July 6: From the ground 1 flower spike appeared just opened and much diseolored; also 1 dead unopened sword. There were 5 spikes bearing several 20-eentimeter nuts, 7 or 8 spikes higher up having no nuts, a discolored opened spike, a dead sword, and 5 or 6 healthy swords. The middle leaves at the top were slightly discolored and a trifle soft rotted. These tips were cut off and 3 of the green swords and central leaves also, for the purpose of opening up the center. About 30 immature nuts were on the ground. The tree was badly diseased, atlhough the rot had not yet reached the heart. One kilogram of salt was placed about the base of the diseased leaves and upper spikes.

July 21: A few more nuts had dropped; otherwise no change.

August 6: Tree was cut down for examination. The salt had no visible effect upon the tissues, and certainly had none in stopping the progress of the disease. The rot had not reached the heart tissues, and all of the young leaves were turning green.

Tree No. 390.

March 11: Eight spikes of nuts and one good sword.

May 28: Same.

June 16: Same.

July 6: Showed one flower spike just opened, the tips chocolate brown in color, and drooping; showed another just opening and the tip chocolate brown; 2 swords. There were 3 spikes of large nuts and 5 or 6 spikes above just dropping their smaller nuts. The middle leaves appeared healthy. One kilogram of salt was plaeed about the base of the upper leaves.

July 21: Had dropped all of its large nuts; otherwise no ehange.

August 6: All spikes were empty and leaves were much yellowed.

Oetober 21: Three dead opened flower spikes; middle leaves turning yellow; hopelessly diseased.

Thus, in this case also, the salt had absolutely no effect in retarding the progress of the disease.

A similar experiment was carried on with the use of copper sulphate crystals, the idea being to poison the diseased areas. It was not

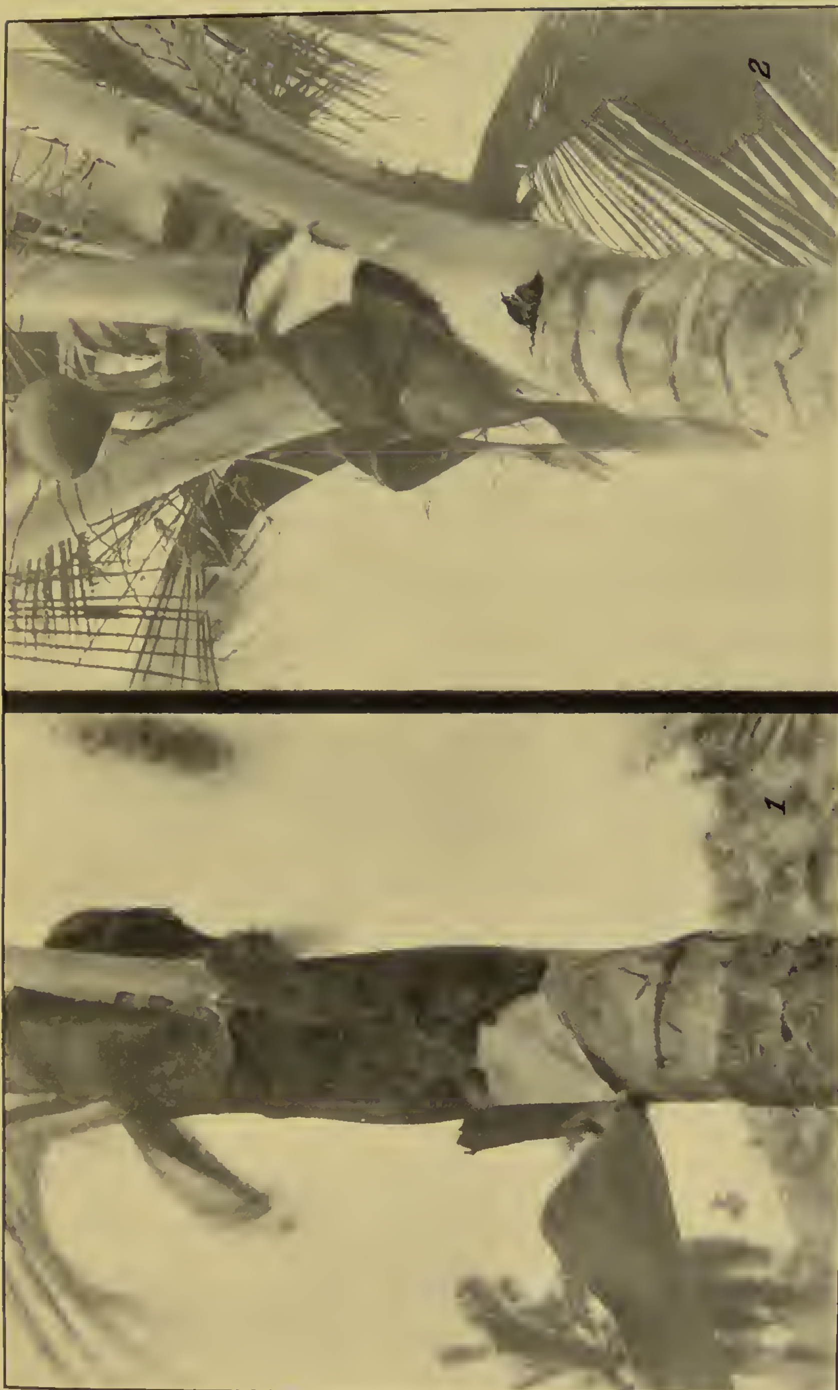


FIG. 1.—DISEASED COCONUT TREE, SHOWING BLACKENED PART OF SHEATH ABOVE THE WHITE, HEALTHY PORTION. FIG. 2.—DISEASED COCONUT TREE, SHOWING DARK WATER-SOAKED SPOTS AT BASE AND SIDE OF PETIOLE.

believed that the copper sulphate would affect seriously the woody tissues with which it would come in contact. Two trees showed the following conditions:

Tree No. 286.

March 11: Nine spikes of nuts and good open flower spike.

May 28: Same.

June 6: Practically the same.

June 29: Appeared unhealthy.

July 6: Had dropped 15 immature nuts; 5 spikes of few nuts; and above were 5 or 6 spikes of no nuts; 3 good swords and good middle leaves. Placed 1 kilogram of copper-sulphate crystals at the bases of the leaves. Rains were so frequent that the crystals were soon dissolved.

July 21: Showed 1 open discolored flower spike.

August 6: Only 2 nuts and many empty spikes on tree; a dead flower spike, opened some time ago, and 1 just opening.

October 21: Central leaves bent over dry and dead; swords dead; many empty spikes; all leaves yellow.

Tree No. 288.

March 11: Sixteen spikes of nuts in good condition.

May 28: Eleven spikes of nuts and 3 good swords.

June 10: General appearance unhealthy.

July 6: Middle leaves healthy and numerous; the second, third, and fourth swords from center were good; fifth and sixth diseased. The seventh and outermost sword was good; the next outward and the adjacent spikes were also good. The 10 or 15 other spikes had no nuts. The lower leaves were yellow.

July 21: Showed from the ground a dead sword and also 2 dead leaves. Applied 1 kilogram of copper sulphate directly to the base of the leaves.

August 8: Very yellow; only 1 green sword left; the tree apparently very far gone.

October 21: Middle leaves bent over, dry, and dead; all the remaining leaves yellow.

Thus from the application of copper sulphate no good result was obtained.

Two trees treated with Paris green, another poison, progressed as follows:

Tree No. 102.

March 7: Seven spikes of nuts and 1 good open flower spike, also 1 good sword; an excellent tree.

May 28: Thirty-six fallen green nuts, 9 spikes of nuts, and 2 good swords.

June 8: Practically the same.

July 8: About 40 fallen immature nuts; 8 good spikes of nuts; 3 spikes with no nuts above the others; 1 good open flower spike on one side and 1 bad open flower spike on the diseased side; 2 good swords and the middle leaves were healthy. About 250 grams of dry Paris green were applied.

July 21: Only 8 nuts left on 4 spikes; a dead sword just breaking open; 1 green sword visible; all leaves green and healthy. Removed 1 spike with 13 3-centimeter nuts, 1 spike with 6 8-centimeter nuts, 1 spike with 1 15-centimeter nut, 1 spike with 1 20-centimeter nut, 1 spike with 1 5-centimeter nut, and 7 spikes with no nuts. The bases of the spikes and of the leaves were well covered with water-soaked areas both on the upper side and on the lower side of the leaf base and spike base. This tree was treated with Paris green, but the spots on the under side could not be caused by

arsenic poisoning, as they were out of reach of the Paris green. Spotting was very abundant. What could cause such a wholesale spotting at such places is a puzzling question. One sword and five leaves were left, and the middle ones besides. The outermost leaves had perfectly clear, undiseased, outside bases; but up toward the strainer were a few water-soaked spots. On the last leaf cut off a water-soaked area appeared as though following the distribution of the Paris green.

August 5: The tree remained about the same; 1 green sword was visible.

October 21: The outer sword was dead, and the middle leaves were also dead. The upper part of the crown was broken and blown over.

Tree No. 105.

March 7: Eleven good spikes of nuts; an excellent tree.

May 28: Same.

June 9: Had 14 fallen immature nuts.

July 8: About 35 fallen immature nuts; 7 spikes holding a few large nuts; above were about 12 spikes bearing no nuts. All the small ones had fallen. The outermost sword was dead, brown, and unopened. There were 4 green, healthy swords. The middle leaves were healthy. The tree had a crown of over 20 leaves, the lowermost circle of which was turning yellow. Applied about 250 grams of dry Paris green at the base of the leaves and below the good spikes.

July 21: There were 4 spikes, 1 brown sword, 2 green swords, and 3 pendent brown leaves. The lower leaves were yellow.

August 5: The central leaves were dry, bent over, and dead. The tree hopelessly diseased.

The treatment with Paris green offers no results different from the others. All applications failed to check the disease. Undoubtedly the criticism will be made that there is great possibility of the poison having hastened the progress of the disease, but no direct evidence appears to support such a supposition. The poison had no definite effect on the tissues with which it came in immediate contact. It is probable that the cutinous covering of the epidermis prevented to a large extent the corrosion, which might be expected. Moreover, the disease on those trees on which the experiments were made did not progress any faster than on adjacent untreated trees of which full records have been kept. It must be borne in mind that the application of these poisons was made rather to note the effect than with any hope of curing the disease. Such treatments have been made by planters in various districts, and the experiment was made to ascertain what possible effect was to be expected. In the light of the knowledge of the arrangement of the leaves at the base of the crown, it is believed to be a physical impossibility for external applications to reach the parts affected by the disease without at the same time seriously injuring the healthy tissues. It is thought that these applications might serve partly to arrest the progress of the infection—to render the condition of development less suitable—but it is certain that they could not absolutely check it.

High hopes have been entertained of another treatment, that of the so-called flaming. So far as public records go this treatment was

first used in Jamaica and subsequently adopted in Cuba. The method consists in setting fire to the crown, either by lighting the dry pendent leaves or by the addition of coal oil. It is desired to create considerable heat in and about the base of the crown and not in the central leaves nor among the upper green leaves. The writer made no experiments with this method as, after the studies on the arrangement of the crown leaves and experiments with the external treatment of other kinds, it was considered to have no practical value. Moreover, an examination of the results of such treatment carried out by several investigators and by planters led to the same conclusion.

The conclusions reached, after carefully examining different trees treated by various workers and watching the progress of many trees so treated during a period of a year, are as follows:

From the arrangement of the crown it is impossible for the heat to penetrate into the inner tissues in sufficient degree to dry out the diseased portion without seriously affecting the growing part of the crown. Any flaming whatever will destroy the lower leaves and all the nuts, so that even if the tree is not killed, at least a year's crop is destroyed. The scorching of the leaves and the charring of the trunk so kills the superficial tissues as to permit the rain to soak in and a subsequent rot to take place. It has been contended by some people applying this treatment to their trees that there was subsequent recovery from the disease, at least to the extent of flower spikes opening out and setting nuts. It should be noted, however, that the tree, while retaining the disease, may send forth new flower spikes and nuts for a period of at least a year after infection has taken place without any treatment having been applied. The writer possesses records of individual trees which show this. In many trees flamed the disease progressed subsequently so that it presented to the writer no evidence of the value of this treatment. As a diseased tree is certain to die if not treated, there can be no error in flaming it; but to try this method with healthy trees in the expectation of warding off infection is not advisable, because (1) there is no evidence that the treatment would succeed, and (2) there is the certainty that the tree would be seriously injured in a way that would make it more susceptible to infection.

PREVENTIVES.

In order to prevent the spread of the disease, it is necessary to destroy its source, or its means of transmission, or the conditions favorable to its development. The absolute destruction of diseased trees, a careful watch for the newly infected cases, and their immediate removal has done much to prevent greater loss in various regions. In Jamaica it is chiefly owing to this care that the bud-rot is not widespread. In some districts in Cuba care has enabled the planters

to maintain successful plantations, while in other districts neglect of the trees has led to their entire destruction.

In 1908 the Government of Cuba appropriated $14,000 to assist in eradicating the disease. Owing partly to the failure to come to an agreement as to the method of work and partly to the lack of some one well enough acquainted with the disease to oversee the project, nothing has as yet been accomplished. Investigators themselves are by no means agreed as to the best method of eradication. The common practice at present is to cut down the diseased tree and, after heaping dry leaves about the crown, to set fire to it. This is fairly satisfactory except that the fallen trunk forms an objectionable obstacle and serves as a breeding place for many insects. Another method recommended is to bury the diseased top with quicklime. Burial presents a task too difficult, if one takes as much of the crown as is necessary to destroy all of the infected parts. Other disinfectants have been recommended for application to the crown as it lies on the ground, but none of these have much value, owing to the difficulty of penetrating to the center of the decayed parts. Personally, the writer is of the opinion that the crown should be cut off as the tree stands and then destroyed. This plan presents no such obstacles as may on the face of it appear, and it has the added advantage that the crown can be entirely burned, leaving no débris to litter the ground or furnish breeding places for insects. The bare trunk left standing would serve as a breeding place for insects to only a very limited extent, since it would remain fairly dry and exposed to all the sunlight and wind—conditions very different from those existing when the trunk lies on the damp ground in the grass and rotting leaves. To be sure, to fell the tree is much the easier method of procedure, but the other way takes only 30 minutes or less with the proper tools. A native can ascend the tree in his bare feet, and with his machete or cutlass quickly lop off all the leaves close down to the heart. If the rot extends below the heart, it would be advisable to pour over its surface a pint or so of coal oil and then set fire to it. The writer has himself ascended trees 15 or 20 meters high by the aid of an electrician's climbers and then removed the leaves with a small, sharp, hand ax. When a planter has only a half-dozen or two dozen trees to destroy, this method seems to be far the best, because it leaves the ground free from any large obstacles. If, on the other hand, several hundred or a thousand diseased trees are to be destroyed as soon as possible, it may be desirable to fell the trees and then burn the tops, afterwards obtaining a two-wheeled rig to draw the logs to one side of the grove where they may be placed in a heap and made the center of a fire, or covered with lime or heaped over with earth. The expense of this work would be excessive for a few trees, but in the case of many it most certainly

would be advisable to carry it out. As a matter of fact it would pay a planter, planning to cut down any great number of trees, to girdle each tree at 1 to 1½ meters from the ground so as to allow the trunk to dry out. Then when it is felled it can be made into very serviceable timber for use on the plantation. It is worthless for use in any constantly moist situation, but is very valuable for siding or beams in sheds and makes especially beautiful pillars.

Besides destroying all sources of infection, it is desirable to reduce to a minimum the number of insects visiting the tree. It has not yet been proved that insects are a means of infection, yet their great abundance in diseased trees leads one to think that they may play an important part in distributing the germs. It is very rare not to find some insects present in an affected crown. The ones most commonly found are the earwigs[1] (*Pyragra buscki* Caud. and *Anisolabis janeirensis* Dohrn), occurring in the damp, rotted, and fungus-infected part of the central leaves some distance above the heart. These same species or closely allied ones have been found by the writer in the diseased trees in Cuba, Jamaica, Trinidad, and British Guiana. There have been a few cases, however, when these forms could not be found, so that they can not be given the entire blame. A few cockroaches[1] (*Leucophaea surinamensis* L., *Blabera fusca* Brunn., and *Periplaneta australasiae* Fab.), are very commonly present in and about damp, decaying portions. Ants may often be found in great numbers, even forming a nest in the crown. A few beetles,[2] such as *Lioderma quadridentatum* Fab., *Cyclonotum flavicorne* Muls., *Ischyrus flavitarsis* Lac., and *Lioderma devium* Muls.; and weevils[2] (*Rhynchophorus palmarum* L.) occur, but seldom in abundance. In the midst of the moist, rotten parts of the tissues centipedes, tree frogs, lizards, small snakes, and rats may very frequently be found. Although it has not been proved that insects carry the disease, it would seem advisable in a seriously infected district to place about the trees, a meter from the ground, bands of cloth 10 centimeters wide, soaked in coal tar, which will prevent the ascent of the tree by any forms of crawling insects. When it is known that rats or any of the larger animals frequent the tops of the trees of any grove it would pay to put a band of galvanized iron about the tree, some 15 centimeters wide and about 1½ meters from the ground. The manner of destroying insects already in the crown of the tree and any flying insects that may come to it is more difficult. In the hope of reducing the number to a minimum the writer undertook to spray a small grove during the course of a year. The experimental work was carried on at a grove about 3 kilometers from Baracoa, which could be reached only by traveling over an extremely rough

[1] Identified by Mr. A. N. Caudell, of the Bureau of Entomology.
[2] Identified by Mr E. A. Schwarz, of the Bureau of Entomology.

road and fording a river. The grove itself was full of underbrush, fallen leaves and logs, and contained some low, marshy places as well as high, dry ground.

Under such conditions it was deemed inadvisable to use an ordinary orchard spraying outfit, as it would be much too cumbersome to move about. A $2\frac{1}{2}$-horsepower gasoline engine and a pump with a $2\frac{1}{2}$-inch cylinder were mounted on a four-wheeled truck only large enough to hold the apparatus. The supply tank was a 200-liter barrel, mounted on two wheels and drawn by hand. Fifty meters of 9.5 millimeter 5-ply hose was taken along and in addition a two-cylinder hand pump. The proposed plan was to apply a mixed germicide and insecticide to the crown. In lieu of some apparatus to raise the spray to the required height—anywhere up to an average of 20 meters and an occasional height of 30 or 40 meters—it was decided to ascend the tree in person, carrying the hose, and then apply the spray to the crown. With the use of iron climbers and leather belt the writer found it possible to do this easily up to 18 meters, and with difficulty up to 20 or 25 meters. Above that height it was, for all practical purposes, impossible, owing to the weight of the hose.

In starting this work it was first necessary to clear out the underbrush and much of the débris littering the ground in order to get about with the spraying apparatus. The entire grove of 145 trees was then sprayed. It was possible to ascend a tree, spray it, and descend in about 15 minutes. An average of about 20 trees per day was the usual result. The grove was sprayed once in February and March, again in June, and a third time in August, 1908. A visit to the grove was made in November, but it was too badly diseased to make another spraying of any value. The following data will give an idea of the results of the spraying; or, perhaps more accurately, shows the progress of the disease in the grove during and after the application of the spraying solution, which consisted of 4–6–50 Bordeaux mixture containing Paris green, 1 part in 3,000:

TABLE V.—*Record of conditions of coconut trees sprayed in 1908.*

Date of inspection.	Number of trees.		Date of inspection.	Number of trees.	
	Newly diseased.	Healthy.		Newly diseased.	Healthy.
Feb. 18	35	145	Aug. 5	64	58
Mar. 11	35	145	Oct. 21	68	45
May 28	23	122	Nov. 20	25	6

This record of the steady spread of the infection during the progress of the spraying is distinctly against any practical value for such work. It may be said, however, that there were conditions

which render the inference to be derived from these experiments not at all so conclusive as it would appear. It is important to inquire whether many, if not all, of the trees which eventually became diseased, may not have been infected before the spraying began. As has already been shown (p. 54), the infection may be present in the crown of a tree and not visible externally. And, further, the solution applied did not adhere evenly, but tended to run together and cohere in patches. When the spray was applied to the coconuts the solution would invariably run down to the under side, where it would dry on in drops. These facts were not particularly prejudicial to the use of the spray, for it was intended and desired to soak thoroughly only the strainer and the tissues at the base of the leaves. The foregoing data show that the spraying did no good in preventing the spread or development of the disease, but there is no definite means of determining the number of insects destroyed. From examining many of the trees in the crown during the course of the year it seemed evident to the writer that the insects were materially reduced, as there were practically none in the crown during the fall, with the exception of ants, of which there were as many as ever.

LABORATORY AND GREENHOUSE STUDIES OF THE DISEASE.

It has been shown in earlier paragraphs that bacterial organisms are able to produce a rotted condition in the heart of the crown of the coconut tree identical with the condition in the typical bud-rot disease as found in the field. It has been shown, also, that certain organisms apparently alike were originally isolated from a typically diseased tree; several cultures of these were inoculated into other coconut trees, and from the successful infections apparently the same organism was reisolated; that a number of these latter cultures were inoculated into other coconut plants, and after producing successful infections the same organisms apparently were again reisolated. In the process of isolation from the diseased tissues many plates were poured, and from these numerous agar-tube transfers were made. Not all of the tubes from any one inoculation proved to be identical, but the greater number turned out to be so. Of all the cultures in the successful inoculations there were two which originally came from the same naturally diseased tree and went through the same process of inoculation, isolation, reinoculation, and reisolation in different trees, and appeared in preliminary tests to be identical in almost every case. Other cultures appeared similar in their reaction in several tests, but these were not compared at any great length. The two original cultures were compared with two cultures from the inoculated trees and two cultures of those isolated from the reinoculated trees. These six cultures were used to make rather

extensive studies of the identity of the organism inoculated, isolated, reinoculated, and reisolated, and for convenience are designated in the culture work as coconut Nos. 1, 2, 3, 4, 5, and 6. By the work described in the following paragraphs the identity of the organisms in these six cultures has been determined and consequently the particular organism which causes the disease is ascertained. In the course of the work such a close similarity of the coconut organism with *Bacillus coli* was observed that comparison of the two organisms was made in most of the cultures.

CULTURAL EXPERIMENTS.

Studies of the Group Characteristics of the Organism.

MORPHOLOGY OF ORGANISM AND COLONY.

The organism causing the coconut bud-rot is a short rod with rounded ends, averaging 1.5 to 1.8 μ in length, and 0.5 μ in width, although in length they may vary from 0.63 to 4.03 μ, and in width from 0.48 to 0.6 μ.. They occur singly, or more commonly in groups of two or three; not infrequently they may be found in chains up to 20 μ in length. The rods are actively motile with several peritrichiate flagella (demonstrated with Löwit's flagella stain) three to four times the length of the organism. Single rods dart about often with a rapid, vibratory motion and sometimes a whirling motion. They also glide from place to place without any apparent vibration. Couples, when in progression, usually move with a bending, waving sort of motion. Occasionally, when in twos or threes, the rods appear almost rigid, but seem to vibrate rapidly at both ends as they glide along. The rods are nonsporulating. Thin-walled capsules are readily apparent when the organism is stained with Löwit's flagella stain. With this stain, also, dense bodies appear within the rods, located either centrally or more or less laterally. The organism does not stain by Gram's method.

On agar plates the organism is variable. The typical surface colonies are slightly raised, white, and semiopaque. Frequently they eventually become lobed or radiate branched, or from the beginning they may be thin and deeply lobed or radiate branched, with a dense nucleus in the center. They are always wet shining; white by direct light and bluish in transmitted light. The surface is always smooth. By transmitted light the thin colony appears homogeneous, but the raised colony or raised margin of the thin colony appears to have streaks more in some parts than in others. In 24 hours the thin colonies may extend 5 or more centimeters on the petri dish, and in 48 hours they frequently cover the dish. The moisture conditions affect the form and density of the colony.

In agar stabs the surface growth is thin and extends across the tube and slightly up the glass. The stab growth is slender and slightly beaded.

On agar streaks the growth is good, irregular, and bordered by numerous tiny white colonies.

On gelatin stabs the surface growth is thin and white. The stab growth is slender and bordered by, or consisting of, many tiny, separate, white colonies.

On gelatin plates the colonies are small, with irregular margins and uneven surface. After several days' growth the colony is usually zonate with alternating ridges and depressions. Savage[1] studied the appearance of *Bacillus coli* on gelatin plates and came to the conclusion that while there was a typical form of colony for this organism, yet there was enough variation in the forms to prevent using this character alone in diagnosing the species. Comparison by the writer of *B. coli* on gelatin plates with the coconut organism shows them to be, for all practical purposes, identical. Usually *B. coli* assumed the zonate form 24 hours later than the coconut organism under the same conditions, but ultimately they appeared indistinguishable.

GROWTH WITH AND WITHOUT AIR.

The fact that the organism grows in the stab inoculations of agar and gelatin and luxuriantly in the closed ends of fermentation tubes is indicative of its ability to grow either in the entire absence of free oxygen or at least when only very little is present.

Experiments were made with large U tubes in which pyrogallic acid and caustic soda were placed in a test tube and inserted in one end of the U and a beef-bouillon culture of the organism was placed in the other end. The ends of the U were sealed by standing them in beakers containing glycerin, and in some of the experiments with glycerin in one beaker and mercury in the other. The fluid used in each case, either glycerin or mercury, rose in the tube as the air was exhausted by the union of the pyrogallic acid and the caustic soda, but it was not possible to say that an absolute vacuum, or rather an entire absence of oxygen, was obtained. In 24 hours the cultures were always well clouded. Similar experiments were made in which it was contrived to insert also in a U tube a test tube containing bouillon, grape sugar, and methylene blue. In no case did this methylene blue lose its color, as would be expected if there were entire absence of oxygen. Similar experiments were made with large straight test tubes. The seal consisted of a rubber stopper smeared with vaseline. Heavy clouding also took place in these tubes, but the methylene

[1] Savage, W. G. Gelatin Surface Colonies of Bacillus Coli Communis. Journal of Pathology and Bacteriology, vol. 9, 1903-4, p. 358.

blue did not fade out. Other experiments were made by cutting off the top of the cotton plug of the inoculated tube, pushing it down below the top of the tube so as to form a cavity, and then putting pyrogallic acid and caustic soda into this space and sealing with rubber stoppers smeared with vaseline. In 24 hours these cultures also clouded.

Ordinary stab cultures were made in fresh nutrient agar, and melted vaseline was inserted with a sterile pipette to cover the agar to a depth of 1 c. c. The agar eventually shrunk and left a space about 3 mm. high between it and the solidified vaseline. A very thin film of growth appeared on the surface of the agar and a fairly good reticulate stab growth was produced, but no more than in agar stab cultures which were not sealed with vaseline.

Another experiment was made with the use of cover glasses. In this experiment 10 c. c. of sterile agar were poured into ordinary petri dishes and the solidified agar inoculated at one point. Sterile cover glasses were then placed over the point of inoculation and pressed down firmly to exclude the air. In 24 hours the growth under the cover glass was distinct and in 48 hours it had reached the edge of the cover glass and eventually spread over the plate.

No more exhaustive experiments have been carried out. It is believed that sufficient has been done to show that the organism will grow well in a reduced amount of oxygen and will grow moderately well in a very small amount of it. It has not been proved that the organism will grow when there is absolutely no oxygen present.

LIQUEFACTION OF GELATIN.

The six coconut cultures were inoculated into beef gelatin and placed in the gelatin box at about 19° C. All remained perfectly firm, until after 11 weeks when No. 1 was somewhat softened. A few days afterwards No. 1 appeared entirely liquefied. Six months after inoculation all remained firm with the exception of No. 1, which was liquefied. The growth on the surface of these tubes first became thin and white, with an irregular margin. The growth along the stab was at first slender and bordered by and consisting of numerous tiny spherical masses. Eventually the growth spread through the medium and over the entire surface.

The foregoing experiment was repeated with the same results. The experiment was again tried and No. 1 liquefied after 16 days, while the others remained solid.

In the work of Longley and Baton [1] for the identification of *Bacillus coli* the cultures containing the suspected organism are incubated in

[1] Longley, F. F., and Baton, W. U. C. Notes on the Determination of B. Coli in Water. Journal of Infectious Diseases, vol. 4, 1907, pp. 397–416.

gelatin at 37° C. for 48 hours, after which they are placed in an ice box to permit hardening. The solidifying of the gelatin is taken as a positive indication of the presence of *Bacillus coli,* and those tubes in which the gelatin did not harden are considered as positively free from *Bacillus coli.*

Cultures of the coconut organism and *Bacillus coli* (from animals) were incubated in gelatin at 37° C. for 48 hours and then placed in an ice box to harden. In six hours all of the tubes, including coconut No. 1, were perfectly firm.

The culture designated as coconut No. 1 was descended from one of many cultures made from the same isolation (505 R). In order to see if they all behaved alike, transfers were made into gelatin from all that were still alive nine months after isolation. There were in all 11 of these cultures from as many colonies. These tubes were incubated at 37° C. and tested daily for liquefaction by removing to an ice box, where they might harden. Seven days after inoculation all showed good growth and were perfectly firm when placed at a low temperature. Ten days after inoculation the check tubes (two) were perfectly firm; four of the cultures were slightly soft; and the remaining seven were in the nature of a thick liquid. Coconut No. 1 was the thinnest of these, but even that was not entirely liquefied.

Attempts were made to plate out coconut No. 1 to ascertain if by any chance it had become contaminated. A variety of colonies was obtained on the plates of plain agar, but transfers from various forms of these colonies to litmus milk, nitrate bouillon, Dunham's solution, neutral red in fermentation tubes, and gelatin all gave the reactions typical of the coconut organism. Platings were also made from the other coconut organisms and from *Bacillus coli* (Theobald Smith, XIV) with exactly the same results. On plain agar the variety of forms was great, ranging from the small, round, iridescent colonies to large, thin, homogeneous, much-branched colonies. Transfers from specific colonies to litmus milk gave the typical reaction for the coconut organism, and platings from these tubes gave in every case on plain beef agar the same variety of forms. In view of this work it does not seem possible that the culture coconut No. 1 was contaminated, but rather, as has been suggested, that it was a modified form of the original organism.

According to the recommendations of the Society of American Bacteriologists six weeks is the time fixed to ascertain the liquefying power of any organism when the cultures are grown in an ordinary gelatin box. *Bacillus coli* and all six of the coconut cultures answer to this test. According to the methods used by some workers and mentioned in the foregoing paragraphs, 48 hours at 37° C. (before placing in an ice box) is sufficient time in which to ascertain the lique-

fying power of an organism. *Bacillus coli* and all six of the coconut organisms answer to this test also. It has been seen, however, that coconut No. 1 if left longer than prescribed in the tests will cause liquefaction. This organism answers to all of the other routine tests for *Bacillus coli*, as do the other coconut cultures. On the other hand, coconut No. 1 differs somewhat in some minor tests. Can, then, *Bacillus coli* and coconut No. 1 be considered identical organisms? Apparently not. It would seem very probable, however, in view of the close similarity of the two organisms that coconut No. 1 was derived from coconut No. 2 (505 S, the organism inoculated into the coconut seedling from which No. 1 was isolated), but had changed its character to some extent. The similarity of coconut No. 1 to the other coconut cultures and to *Bacillus coli* will be brought out more clearly in subsequent pages.

PRODUCTION OF ACID AND GAS IN DEXTROSE.

In one series of experiments the average gas production in 10 days in the closed end of fermentation tubes was 24 millimeters and the average titration was +25 on Fuller's scale. As will be seen, however, in the citation of various titrations and gas production, there is considerable variation. Table VI gives the acidity ascertained on various cultures. The amounts are typical of many other titrations.

TABLE VI.—*Acid production by coconut cultures Nos. 1 to 6 and Bacillus coli in various media at 22° C.*[1]

Culture.	Medium 4193: 1 per cent peptone +2 per cent dextrose.	Medium 4192: Dunham's solution+2 per cent dextrose.				Medium 4268: Dunham's solution+1 per cent dextrose.			
	12 days.	6 days.	23 days.	31 days.	55 days.	20 hours.	5 days.	19 days.	47 days.
Coconut 1	....	+26	+14	+14	+20	....	....	....	....
Coconut 2	....	+27	+14	+14	+16	....	....	....	....
Coconut 3	....	+28	+14	+14	+16	....	....	....	....
Coconut 4	....	....	+14	+14	+16, +16	....	....	....	....
Coconut 5	+18	+26	+14	+14	+16.5	+9	+11	+13	+32, +32
Coconut 6	....	+27	+14	+14	+16	....	....	....	....
Bacillus coli	....	+24	+15	+15	+16	....	+11	+14	+36, +36
Check	....	+ 6	+ 4	+ 4	+ 3	+3	....	....	....

[1] Distilled water used in these 3 media.

In a dextrose solution in distilled water, originally +5, the culture became +11.5, showing an acid production but not so great (nor so good a growth) as in the presence of peptone also.

In dextrose plus KNO_3, originally +5, the culture became +8, this also showing less growth (and less acid) in the absence of peptone.

Cultures in peptone plus rosolic acid made good growth but did not affect the color. Cultures in peptone plus rosolic acid plus dextrose clouded well and changed from the pink color of the check to a sort of orange yellow, thus indicating the production of an acid.

Cultures in peptone plus neutral red grew well and changed the color of the medium to an orange yellow, in this case showing a slight production of alkali. In cultures containing peptone plus neutral red plus dextrose the growth was good and the color changed to a magenta, thus indicating the production of an acid.

A series of cultures was made in Dunham's solution colored with litmus, some of the tubes containing grape sugar and others without any sugar. Those containing the sugar in three days became well reddened and partially bleached. Those without the grape sugar became well clouded and showed good growth, but did not change in color from the check.

The amount of gas produced in media containing dextrose is shown in Table VII.

TABLE VII.—*Amount of gas (in millimeters) produced by various cultures in different media, experiments 1, 2, 3, and 4.*

Culture.	Peptone plus dextrose at 22° C.										Beef bouillon plus dextrose at 37° C.		
	Experiment 1, Dec. 8-14, 1909.		Experiment 2, Dec. 13, 1909, to Jan. 3, 1910.			Experiment 3, Jan. 17-27, 1910.					Experiment 4, Mar. 16-19, 1910.		
	2 days.	6 days.	1 day.	5 days.	21 days.	2 days.	4 days.	5 days.	7 days.	10 days.	1 day.	2 days.	3 days.
Coconut 1	5	27	0	0	......	1	......	32	45	56	......	......	......
Coconut 1 a	5	46	......	......	......	7	......	36	49	......	......	......	......
Coconut 2	5	13	0	8	2	11	21	......	......	......	45	51	55
Coconut 2 a	5	14	......	......	......	11	......	......	......	......	......	......	......
Coconut 3	0	0	......	......	......	11	......	26	28	26	39	44	46
Coconut 3 a	0	0	0	0	0	11	......	29	30	30	51	56	57
Coconut 4	0	0	......	......	......	0	......	29	30	......	46	52	53
Coconut 4 a	0	0	0	0	0	0	......	0	0	0	44	47	47
Coconut 5	5	15	0	10	4	9	......	20	23	27	41	46	46
Coconut 5 a	5	17	......	......	......	0	......	17	19	14	41	49	53
Coconut 6	0	0	0	0	0	9	......	24	24	24	46	49	50
Coconut 6 a	0	0	......	......	......	15	......	29	29	28	......	......	......
Bacillus coli	......	......	......	......	......	......	......	......	......	......	50	52	48
B. coli a	......	......	......	......	......	......	......	......	......	......	......	47	50

The failure of some of the cultures to produce gas in experiments 1 and 2 while they readily produced it in experiments 3 and 4 is rather striking and not readily explainable. Occasionally, in subsequent experiments not noted here, a similar failure of a certain culture to produce gas in dextrose has occurred. From further cultures of descendants of such a culture, however, it is evident that the same

organism has been retained throughout, and that there has been no discernible contamination of the tubes.

PRODUCTION OF ACID AND GAS IN LACTOSE.

Titration of cultures in lactose fermentation tubes showed coconut No. 4 to be +15 and No. 6 +15 on Fuller's scale after growth for 15 days. No checks were titrated at this time, but they usually ran about +5.

In fermentation tubes containing 1 per cent peptone plus 1 per cent lactose in 24 hours no gas was shown. After five days at 22° C. the showing was as follows: Tube 1 a, 34 mm.; tube 2 a, 20; tube 3, 26; tube 4, 20; tube 5 a, 27; tube 6 a, 24.

After 15 days No. 5 a had produced 28 mm. of gas. In 21 days No. 6 a showed only 22 mm. of gas. Evidently the gas production had ceased and that already formed was being partly absorbed.

Table VIII shows the results of a similar experiment in peptone plus lactose at 22° C.:

TABLE VIII.—*Amount of gas (in mm.) produced by various cultures in experiment with 1 per cent peptone plus 1 per cent lactose, at 22° C., February 4 to 18, 1910.*

Culture.	1 day.	3 days.	7 days.	10 days.	14 days.
Coconut 1	0	0	6	12	21
Coconut 2	Tiny bubbles.	9	2	Tiny bubbles.	Tiny bubbles.
Coconut 3	0	0	0	0	0
Coconut 4	0	0	0	0	0
Coconut 5	0	11	8	4	1 large bubble.
Coconut 6	0	0	0	0	0

In the lactose as in the dextrose the ability of the organism to produce gas appears to vary. In the first experiment all of the cultures formed gas; and in the later experiment only three of them, and they apparently with difficulty. The fact that the organism can effect this change, even though it does not always do so, is of value in determining its characteristics. No experiments were carried out using beef bouillon with lactose. In general, the acid production in lactose seems to be less than in dextrose; and the gas formation also, at least in a peptone medium, seems to be effected with greater difficulty than in dextrose.

PRODUCTION OF ACID AND GAS IN SACCHAROSE.

Cultures in peptone plus cane sugar titrated after five days at 22° C., coconut No. 1, +14; No. 5, +13. No check was titrated at this time, but they usually were about +5. The following table shows the gas production:

TABLE IX.—*Amount of gas (in mm.) produced by various cultures in 1 per cent peptone plus 1 per cent cane sugar at 22° C., experiments 1 and 2.*

Culture.	Exp. 1: Dec. 8–28, 1909.				Exp. 2: Feb. 4–20, 1910.				
	1 day.	2 days.	10 days.	20 days.	1 day.	3 days.	7 days.	10 days.	14 days.
Coconut 1	0	5	40	50	0	13	40	50	59
Coconut 1 a	0	5	70	55	......	......	......	......	......
Coconut 2	(1)	5	13	12	5	11	10	5	(2)
Coconut 2 a	(1)	5	13	15	......	......	......	......	......
Coconut 3	0	0	0	[3] 0	0	0	0	0	0
Coconut 3 a	0	0	0	[3] 0	......	......	......	......	......
Coconut 4	(1)	0	0	0	0	0	0	0	0
Coconut 4 a	0	0	0	0	......	......	......	......	......
Coconut 5	(4)	5	15	20	5	14	10	13	15
Coconut 5 a	(4)	5	17	15	......	......	......	......	......
Coconut 6	0	0	0	(2)	0	0	0	0	0
Coconut 6 a	0	0	0	(2)	......	......	......	......	......

[1] Tiny bubbles.
[2] One large bubble.
[3] Various tubes of this culture previously produced gas in cane sugar.
[4] Small amount.

Here, as in the case of dextrose and lactose, is a failure on the part of certain cultures to produce gas. Coconut Nos. 3, 4, and 6 seem to have less power in this direction than the other cultures. In dextrose and lactose media these cultures sometimes failed and sometimes did not. In the only experiments carried out with cane sugar they failed.

GROWTH IN NITRATE BOUILLON.

Cultures were made in nitrate bouillon, and within two days, tests for nitrites with starch, potassium iodid, and sulphuric acid showed the presence of nitrites, thus indicating the ability of the organism to reduce the nitrates. This test has been repeated many times and in no case did any of the cultures fail to give a good reaction.

COLOR PRODUCTION.

The coconut organism does not produce any pigment. On plain agar the growth is white in direct light. Where the growth is thin on agar plates the colonies in transmitted light are bluish. On potato cylinders there may be a slight yellow color to the growth. On media containing neutral red the growth is pink, eventually fading to orange yellow, but that is undoubtedly due to the presence of the neutral red. On Kashida's litmus-lactose agar (p. 119) the colonies are first slate blue, then red. On Endo's fuchsin agar (p. 88) the growth is bright red, or, at a certain stage, white in direct light and iridescent in transmitted light. All of these colors, however, are doubtless due to reactions in the medium and are not direct products of the bacterial growth.

GROWTH ON STARCH MEDIA.

Experiment 1.—Both the coconut organism and *Bacillus coli* were grown on potato cylinders and after 18 days were tested with an iodin potassium iodid solution for amylodextrin as follows:

Coconut No. 1 showed distinctly the red purple on the addition of Lugol's iodin. Some blue fragments indicated that not all the starch had been converted.

No. 5 showed a good red-purple reaction and also abundance of blue.

Bacillus coli showed red purple almost throughout the cylinder, there being only a few fragments of blue.

Check-potato cylinders showed only a bright Prussian blue on the addition of Lugol's iodin.

Experiment 2.—A nutrient solution consisting of 1 per cent peptone, 2 per cent dextrose, and 0.1 per cent cornstarch was made up, and a number of tubes were inoculated with the coconut and *Bacillus coli* cultures. After incubation for 17 days at 22° C. the tubes were tested for the presence of starch. As it was found in preliminary tests that a small amount of Lugol's iodin gave only a transitory blue, the exact amount used for the test was determined. It was also found that a tube showing a good test for one minute or five minutes might be entirely colorless after a longer time. One minute was selected arbitrarily as the limit of time for the color reaction to persist. It was found that from four to five drops of the iodin solution were necessary to give the Prussian blue starch reaction in a check tube and have it persist for one minute. Among the culture tubes it was found that, on the average, about 80 drops of the iodin solution were necessary to make the dark color persist one minute. While the test gave the bright blue in the check tubes, in all the culture tubes the color reaction was a red purple, or on long standing and adding more iodin a red or rich brown color. There was no evidence of the blue starch reaction in the culture tubes except on examination with the microscope. By this means blue particles could be found, but only in small quantities. This experiment demonstrated clearly that the starch is affected by the organism and in this particular case using a dextrose-peptone medium nearly all of the 0.1 per cent starch was converted.

Experiment 3.—Cultures of the coconut organism and *Bacillus coli* were made in three different media as follows: (1) Two per cent peptone plus 0.05 per cent NaCl plus 0.1 per cent potato starch; (2) the same with the addition of litmus; (3) a third medium similar to the first with the addition of dextrose. The purpose of this experiment was to ascertain if the action of the starch was independent of any sugar present. Litmus was added to the first medium to serve as a check to indicate the presence or absence of acid formation. If any acid were formed it would be from the starch. No titration was made of these cultures. It was assumed from other experiments

that an acid would be formed in the presence of the sugar. The results in growth are as follows:

After one day at 37° C: Series 1 has all tubes moderately clouded but no gas; series 2, same with no change in color of litmus; series 3, has all tubes well clouded and abundant gas production.

After two days at 37° C: Conditions same in each tube.

After four days at 37° C: Series 1, all moderately clouded and thin films on surface; no gas formed. Series 2, all moderately clouded with thin film on surface; litmus, shows no signs of reddening nor of bleaching. Series 3, all well clouded with small amount of gas.

Test for starch in series 1: Checks with 26, 30, and 41 drops of iodin solution all showed blue-starch reaction. Culture No. 1 showed very transitory blue with 5 and up to 40 drops of iodin; not tested higher. Culture No. 2 with 61 drops produced a dark purple blue, persistent for at least 1 minute. Culture No. 3, up to 20 drops showed no color; 30 and 40 drops caused a slight tinge of purple. Culture No. 4, from 5 to 30 drops caused a very transient blue; 40 drops produced a blue lasting 5 seconds. Culture No. 5, 15 drops produced no color whatever; not tested higher. Culture No. 6, from 5 to 30 drops caused a very transient blue; 40 drops produced a blue lasting 5 seconds. *Bacillus coli*, as high as 40 drops were added without producing any starch reaction or other color.

Series 2 was not tested owing to the presence of the litmus.

Test for starch in series 3: Checks—(a) transient blue with 10, 15, and more drops; (b) same; (c) transient purple with 10 drops; (d) transient blue with 10 and 15 drops; with 20 drops lasts 5 seconds. Culture No. 1 showed transient red purple with 10 drops; with 15 drops color lasts 1 minute. Culture No. 2 showed dark purple blue with 24 drops. Culture No. 3 showed transient red purple, same as No. 1. Culture No. 4 showed blue at least 30 seconds with 10 drops; blue black and not purple with 15 drops. Culture No. 5 showed bright blue with 10 drops; dense blue black with 15 drops. Culture No. 6 showed purple with 15 drops of iodin. *Bacillus coli* showed purple with 10 drops, lasting slightly longer than with No. 3.

In series 1 no distinct amylodextrin test was obtained, the occasional purple blue being only what may occur in ordinary starch tests. If the iodin solution is added to starch the first reaction is a bright Prussian blue, but this on standing ultimately often becomes purple. The distinct red-purple reaction formed in some of the tests of series 3 is the typical reaction and one quite distinctive. This experiment tends to show that the diastase, if it is a diastase that produces the change from starch to amylodextrin, is produced in the presence of a sugar and not in its absence.

Experiment 4 a.—Cultures were made on slant potato-starch jelly tubes and incubated at 37° C. In 24 hours coconut cultures Nos. 3 and 4 showed good narrow, dry streaks. The other cultures showed no growth. In three days all the tubes but coconut No. 5 showed good growth on the surface of the slant. No 3 was tested with Lugol's iodin solution, but showed no signs of amylodextrin. No. 1 showed a slight red-purple coloration. None of the other cultures showed any signs of the amylodextrin reaction; all were bright blue.

Experiment 4 b.—This experiment was repeated and the tubes were tested, after growth, for five days at 37° C. In the *Bacillus coli* tube the application of the iodin solution indicated the change of amylodextrin over the surface of the slant and around the outside of the cylinder next to the glass halfway down the tube. This change did not extend to any appreciable depth. In the coconut-culture tubes the slightest indication of the amylodextrin was seen on applying the iodin solution. Practically all the color in these tubes was the bright Prussian blue characteristic of the pure starch.

Experiment 4 c.—Other cultures were made of coconut No. 5 and *Bacillus coli* upon slant jelly. Two tubes of each culture were inoculated for 23 days at 37° C. At the end of that time each tube had about 1 c. c. of a watery fluid, apparently from the disintegration of the starch; there was good growth, and in the case of *Bacillus coli* the jelly was somewhat browned. Examination of the cultures showed results as follows:

Coconut No. 5: The jelly is soft on the surface and sides, firm beneath. The test shows abundant red-purple coloration. About three-fourths of the cylinder was unaffected by the organism and showed a good blue starch reaction.

Coconut No. 5 a: Same as No. 5.

Bacillus coli: The jelly is very soft. The test shows nearly all red purple with only a small amount of blue reaction.

Bacillus coli a: Like Nos. 5 and 5 a in every respect.

Experiment 4 d.—Another series of cultures was made in which the six coconut organisms and four strains of *Bacillus coli* were used. After incubation for two weeks at 37° C. the tubes showed the following conditions:

Coconut No. 1: No sign of growth on slant; no amylodextrin shown by test. Nos. 2, 3, 4, and 5: Good slightly yellow streak; no amylodextrin. No. 6: Good distinctly yellow streak; no amylodextrin.

Bacillus coli (Hitchings) No. 1: Good yellowish growth over entire surface; liquefaction along sides; large amount of amylodextrin shown by test. *Bacillus coli* (Hitchings) No. 2: Narrow slightly yellow streak; some water; small amount of amylodextrin. *Bacillus coli* (VI–11–V–09) No. 1: Good yellowish growth over entire surface; abundant water along sides; definite but small amount of amylodextrin. *Bacillus coli* (VI–11–V–09) No. 2: Same growth and amount of amylodextrin. *Bacillus coli* (B. A. I.) No. 1: Same growth; small amount of amylodextrin. *Bacillus coli* (B. A. I.) No. 2: Good growth on surface and some water; no amylodextrin. *Bacillus coli* (Theobald Smith, XIV) No. 1: Narrow streak; very small amount of amylodextrin. *Bacillus coli* (Theobald Smith, XIV) No. 2: Narrow brown streak; small amount of amylodextrin.

Summary of starch-media experiments.—The results of these experiments may be briefly summarized as follows:

Experiment 1. Potato cylinders, amylodextrin formed.
2. Peptone-dextrose cornstarch solution, amylodextrin formed.
3. Peptone-potato starch solution, amylodextrin formed.
Peptone-dextrose potato-starch solution, no amylodextrin formed.
Peptone-potato starch with litmus, no acid formed; not tested for amylodextrin.
4 a. Starch jelly, no amylodextrin.
4 b. Starch jelly, amylodextrin formed.
4 c. Starch jelly, amylodextrin formed.
4 d. Starch jelly, no amylodextrin formed.

These experiments demonstrate the ability of the coconut organism as well as *Bacillus coli* to change starch into amylodextrin, although this power appears to be variable. *Bacillus coli* is, in general, more constant in this power and usually more effective. Contrary to the foregoing results of the author in regard to *Bacillus coli*, Savage[1] states that true *Bacillus coli* does not ferment starch.

PRODUCTION OF ACID AND GAS IN GLYCERIN.[2]

Cultures in peptone plus glycerin after growth for 5 days titrated +10 for cultures of coconut No. 3 and +11 for coconut No. 4. The gas production in this medium is shown in the following table:

TABLE X.—*Amount of gas (in mm.) produced in 1 per cent peptone plus 1 per cent glycerin at 22° C., February 4 to 20, 1910.*

Culture.	1 day.	3 days.	7 days.	10 days.	14 days.
Coconut 1	0	0	0	0	0
Coconut 2	One bubble.	11	13	10	10
Coconut 3	0	0	0	0	0
Coconut 4	0	0	0	0	0
Coconut 5	Few bubbles.	10	11	11	10
Coconut 6	0	0	0	0	0

In Table X it is seen that cultures Nos. 2 and 5 have produced gas in the glycerin medium while the others have failed. This experiment was repeated to determine if the same result would again be obtained at a higher temperature. The results appear in Table XI.

[1] Savage. W. G. The Characters of the Bacillus Coli as an Indicator of Excretal Contamination. Lancet, London, vol. 168, Feb. 4, 1905, p. 287.

[2] Giddings, N. J. A Bacterial Soft Rot of Muskmelon, Caused by Bacillus Melonis, n. sp. Bulletin 148, Vermont Agricultural Experiment Station, January, 1910, p. 400. The author reports 6 per cent of gas formed in nutrient broth cultures of *Bacillus coli* containing 2 per cent glycerin.

TABLE XI.—*Amount of gas (in mm.) produced by various cultures in 1 per cent peptone plus 1 per cent glycerin at 37° C., June 18–27, 1910.*

Culture.	2 days.	3 days.	4 days.	7 days.	9 days.
Coconut 1	([1])	([1])	([1])	([1])	([2])
Coconut 2	[3] 36	([4])	40	41	40
Coconut 3	([1])	([1])	([1])	([1])	([2])
Coconut 4	([1])	([1])	([1])	([1])	([2])
Coconut 5	[3] 32	([4])	32	32	30
Coconut 6	([1])	([1])	([1])	([1])	([2])
Bacillus coli (Hitchings)	([1])	([1])	([1])	18	21
Bacillus coli (B. A. I.)	([1])	± 5	11	27	32
Bacillus coli (XIV)	([1])	([1])	([5])	29	32
Bacillus coli (VI-11-V-09)	([1])	± 5	17	27	27
Check	([6])	([6])	([6])	([6])	([6])

[1] No gas; barely clouded in closed end.
[2] No gas; thinly clouded in closed end.
[3] Moderately clouded in closed end.
[4] About as before.
[5] Two large bubbles of gas.
[6] Clear in both ends.

GROUP NUMBER OF THE COCONUT ORGANISM.

It has been found in some of the preceding experiments that the six coconut cultures were neither constant nor identical in their reaction in every medium. In the case of liquefaction of gelatin, No. 1 liquefied the gelatin after several weeks, the others not even after six months. In dextrose medium all produced gas; No. 1 more than the others. In lactose medium all produced gas sometimes, but not always. In media containing saccharose as the only carbohydrate all the cultures produced gas at one time or another. In nitrate bouillon all the cultures reduced the nitrates to nitrites. As to color of the colonies, all may be considered nonchromogenic. As to growth on starch media, the coconut organisms have been definitely proved to have a variable effect on the starch, sometimes converting it rapidly into amylodextrin but more frequently feebly or not at all. In glycerin media the production of gas occurred only in Nos. 2 and 5. Doubtless the other strains might also produce gas if grown under the right conditions. It seems more reasonable to believe this than not for this reason: Nos. 2 and 5 are cultures isolated from two different coconut seedlings which were inoculated with Nos. 1 and 4, respectively, and 1 and 4 have here behaved alike. Moreover, Nos. 2 and 5, after being isolated, were reinoculated into two different coconut seedlings and from them were derived cultures Nos. 3 and 6, respectively, these being identical in this case with Nos. 1 and 4. That in such a set of experiments Nos. 3 and 5 are different species or varieties is an idea scarcely conceivable. Granted that this organism represented by the six strains is capable of producing gas in glycerin media, it then appears that for this organism the following formula according to the chart of the Society of American Bacteriologists must be used: 222.1111021. The identity of this formula with that of *Bacillus coli* is at once apparent. Under these circumstances many more biological features of the coconut organism must be ascertained in order to distinguish it from or to identify it with the colon organ-

ism. As much work has been done by various investigators toward ascertaining a ready means of identifying *Bacillus coli*, it has been deemed advisable to consider these special means of identification before taking up the many miscellaneous biological features commonly discussed in this sort of work.

SPECIAL TEST REACTIONS FOR THE IDENTIFICATION OF THE ORGANISM.

Various bacteriologists, working in connection with public boards of health or independently, have adopted routine methods for determining the presence of *Bacillus coli* in drinking water or elsewhere. In some cases these methods are considered by their users as sufficient to ascertain definitely the presence or absence of this organism. In other cases the probabilities are that the organism in question is *Bacillus coli* or some closely allied form. The tests made consist of only one or two or of several reactions. The adoption of the special methods here will be of service, not only in characterizing the coconut organism, but also in ascertaining any differences that there may be between it and *Bacillus coli*.

Of those reactions described in the following pages Dr. Theobald Smith [1] claimed that the production of gas to the amount of 40 to 70 per cent in dextrose media demonstrated the presence of the colon group of bacteria, the hog cholera group, *Bacillus lactis aerogenes*, and Friedlander's bacillus. In order to determine from among these various bacteria the true *Bacillus coli*, Dr. Smith suggested[2] that the following reactions were sufficient: Growth on gelatin in the form of delicate bluish or more opaque whitish expansions with irregular margins; actively motile when examined in a hanging drop from young surface colonies taken from the gelatin plates; coagulation of milk within a few days; growth upon potato either a rich, pale or brownish yellow deposit, or merely glistening, barely recognizable; also gives a distinct indol reaction.

Behavior of the organism in the fermentation tube must conform to the following scheme:

Variety A: One per cent dextrose bouillon at 37° C. Total gas about one-half the volume of the closed arm. Proportion of hydrogen to carbon dioxide about 2:1. Reaction strongly acid.

One per cent lactose bouillon. As in dextrose bouillon with slight variations.

One per cent saccharose bouillon. Gas production slower than in the preceding. Total gas finally about two-thirds. Proportion of H to CO_2 nearly 3 to 2. The final reaction in the bulb may be slightly acid or alkaline according to the rate of gas produced.

Variety B: The same as variety A, except that in saccharose bouillon neither gas nor acid is formed.

---

[1] Smith, Theobald. The Fermentation Tube. The Wilder Quarter-Century Book, 1893, p. 229.

[2] Smith, Theobald. Notes on Bacillus Coli Communis and Related Forms. American Journal of Medical Science, vol. 110, 1895, pp. 283–302.

Dr. B. H. Stone [1] describes a rapid method of identifying *Bacillus coli* in water. A fermentation tube is filled with 2 per cent glucose bouillon and this is inoculated with 1 cubic centimeter of the water to be examined and grown 24 hours at 38° C. If from 25 to 70 per cent of gas is formed in the closed arm *Bacillus coli* is probably present. From those tubes which produce this amount of gas transfers of 0.5 c. c. are made to tubes containing 10 c. c. of neutral broth to which has been added 0.3 c. c. of Parietti's solution, and the tubes are grown 24 hours at 38° C. From those tubes containing Parietti's solution transfers of 0.5 c. c. are made to fresh fermentation tubes, and if gas is produced as before there is reasonable certainty that the organism is *Bacillus coli*. Further confirmation is obtained by ascertaining the gas formula, that of the colon group being $H:CO_2::2:1$.

Further transfers from the supposed *Bacillus coli* may be made into gelatin stab cultures, into litmus milk, and into Dunham's solution for indol. Also the morphology may be ascertained. These reactions are considered by Dr. Stone sufficient to verify the identification of *Bacillus coli*.

Drs. F. F. Longley and W. U. C. Baton [2] have published their routine method for identifying *Bacillus coli* in water, as follows:

1. Incubation in ordinary dextrose broth and fermentation tubes at 40° C. for 24 hours. From those tubes showing gas within 24 hours transfers are made to litmus-lactose-agar plates.

2. The litmus-agar plates are incubated at 40° C. for 18 to 26 hours. From those colonies which appeared red on these plates transfers are made to agar slants.

3. Agar slants are incubated for 24 hours at 40° C. Those slants which have the typical cultures characteristic of *Bacillus coli* are not examined microscopically. Atypical colonies are examined before discarding.

4. A. Those agar slants which show typical cultures are transferred to dextrose broth fermentation tubes and incubated at 40° C. for 24 hours. The absence of gas is considered negative. The quantity of gas present and the proportion of CO_2 are not determined.

4. B. Milk. Transfers to milk cultures are incubated at 40° C. for two days and examined daily for coagulation and digestion of the casein. Coagulation indicates *Bacillus coli*.

4. C. Nitrate broth cultures. Incubated at 40° C. for two days and then tested for nitrites. The presence of nitrites indicates *Bacillus coli*.

4. D. Peptone broth. Cultures incubated for three days at 40° C. and tested for indol. Presence of indol indicates *Bacillus coli*.

---

[1] Stone, B. H. A Rapid Method of Detecting Bacillus Coli Communis in Water. American Medicine, vol. 3, Jan. 25, 1902, p. 154.

[2] Longley, F. F., and Baton, W. U. C. Notes on the Determination of Bacillus Coli in Water. Journal of Infectious Diseases, vol. 4, 1907, pp. 397–416.

Dr. W. G. Savage [1] studied the following points in identifying *Bacillus coli:* Motility, liquefaction of gelatin, type of colony on gelatin, indol production, acid production, milk coagulation, and fermentation of dextrose and lactose.

Dr. D. Rivas [2] states that the usual method for identifying *Bacillus coli* is as follows:

Plating out in Wurtz's litmus-agar plates with 1.5 to 2 per cent Parietti's solution. Examined after 24 hours' incubation at 37° C. All the pink colonies are isolated and transferred to sugar media for fermentation.

Transfers are made to Dunham's solution to test for indol. Also to nitrate bouillon to test for reduction of nitrates to nitrites.

Further transfers are made to gelatin tubes for liquefaction or nonliquefaction.

Dr. J. J. Kinyoun uses Endo's fuchsin agar for the determination of *Bacillus coli.* His method of making up the medium is one that he considers as furnishing a very distinctive test, as follows:

Take 2 liters tap water, 40 grams Liebig's meat extract, 40 grams Witte's peptone, 20 grams sodium chlorid, 160 grams agar flour. Put into a tall beaker and steam for three hours. Let settle over night. Cut off dirty part and throw away. Melt the remainder and neutralize to phenolphthalein. Add 4 c. c. of $\frac{N}{1}$ hydrochloric acid. Steam one hour. This forms the stock, which should be clear. The crux of the whole formula lies in the following: Take 200 c. c. of this hot stock and add to it 2 grams of lactose. Then add 2 c. c. of a solution of basic fuchsin (half-saturated solution) and 10 c. c. of fresh sodium-sulphite solution (5 grams to 100 c. c. of water). Divide into eight lots of 25 c. c. each to form the trial lots. Make up a 10 per cent solution of sodium carbonate, and add of this to the trial lots, in varying amounts, as follows: 0.01 c. c., 0.02 c. c., 0.03 c. c., 0.04 c. c., etc. Pour into large plates, cool, and streak for colon, typhoid, etc. Incubate 24 hours at 37° C. The standard of alkalinity to be used on the remainder of the stock is that of the plate which has given the most characteristic results. Fill and set away the stock in 100 c. c. portions in bottles plugged with cotton. As there is much water of condensation, the agar is hardened in the plates uncovered in a clean place. Air germs (exclusive of molds) seldom grow on it.

These points are stated to be the most essential in the identification of *Bacillus coli.*

The growth of the coconut organisms in various other media is described on the following pages.

DOLT'S SYNTHETIC MEDIUM NO. 1.

On slant cultures in Dolt's medium a good pink growth appeared within 24 hours, and the agar became partly reddened. Evidence of gas appeared in tubes of coconut No. 2 and *Bacillus coli.* Repetition of this experiment gave exactly the same result with the exception of no gas production. The growth along the streak was for the most

[1] Savage, W. G. The Characters of the Bacillus Coli as an Indicator of Excretal Contamination. Lancet, London, vol. 168, Feb. 4, 1905, p. 284.

[2] Rivas, D. B. Coli Communis, "The Presumptive Test," and the Sewage Streptococci in Drinking Water. Journal of Medical Research, vol. 16, 1907, pp. 85-98.

part wet shining, smooth, and with raised margins. Near the top the growth consisted of more or less isolated colonies. In tube No. 1 a subsequent tendency to bleaching of the litmus appeared. In all the tubes the litmus was first reddened.

This medium is made up as follows:[1]

	Cubic centimeters.
Purified agar (3 per cent solution)	500
Glycerin .....5 grams; Ammonium phosphate..1 gram; Distilled water	500

NaOH was used to neutralize, and 1 per cent lactose added just before sterilization. Litmus was added in sufficient quantity to make a good blue color.

NEUTRAL RED USED IN VARIOUS MEDIA.

The use of neutral red in differentiating *Bacillus coli* from other species has been widely recommended. A few other organisms behave in a similar way, but the reaction at least differentiates a group of organisms if not a single one. A useful way to use neutral red in determining *Bacillus coli* is in fermentation tubes. This method and others are here described.

TABLE XII.—*Growth of coconut and Bacillus coli cultures of March 15, 1910, in fermentation tubes, using neutral red with 1 per cent dextrose and 1 per cent peptone solution in river water, incubated at 37° C.*

Culture.	3 days.	6 days.	8 days.	15 days.
Coconut 5	Greenish in closed end; 30 mm. gas.	Pink in open end; greenish-yellow in closed end.	Pale pink in closed end; bright pink in open end; 33 mm. gas.	No change.
Coconut 5 a	Pink in both ends; 25 mm. gas.	do	Greenish yellow in closed end; pink in open end; 28 mm. gas.	Do.
Bacillus coli	Pink in both ends; 26 mm. gas.	Pink in both ends	Pink in both ends; 32 mm. gas.	Do.
Bacillus coli a	Greenish in closed end; 24 mm. gas.	Pink in open end; bleached in closed end.	Pale pink in closed end; bright pink in open end; 27 mm. gas.	Do.

The greenish-yellow color seen in tubes 5 and *Bacillus coli* a of this experiment is typical of the neutral-red reaction. Tubes 5 and 5 a, *Bacillus coli* and *Bacillus coli* a, derived, respectively, from 5 and *Bacillus coli*, did not show the same reaction. As the two tubes each from different cultures were made under the same conditions from the same tubes, this difference in results suggests an unreliability in the reaction. Further work, however, tends to show that the greenish-yellow or canary-yellow color is generally present. In other media it is demonstrated more clearly.

[1] Dolt, Maurice L. Simple Synthetic Media for the Growth of Bacillus Coli and for Its Isolation from Water. Journal of Infectious Diseases, vol. 5, 1908, p. 625.

Cultures with peptone and dextrose plus neutral red were made in ordinary test tubes. The check was an orange-red color. The culture tubes became in four days a magenta. In nine days they were a deep magenta with the exception of tube No. 1, which had paled to an orange red.

The change to an orange-red color may be accounted for by the production of ammonia, a small amount of which is found in peptone cultures of this organism. (See p. 93.)

This experiment was repeated with the same results. The color of the culture solutions (except No. 1, almost bleached) in transmitted light corresponds to Tyrian rose, tint No. 3, Répertoire de Couleurs, Publié par la Société Française des Chrysanthémistes.

Cultures without dextrose were made. In four days these still remained a pink color, though a trifle paler than the check. In nine days all were orange red.

This experiment was repeated with the same result. The color of the culture corresponded to reddish terra cotta, tint No. 2, Répertoire de Couleurs. The check tube very closely corresponded to the reddish old rose, tint No. 4.

In none of these cultures in peptone solutions with neutral red in straight test tubes was there any of the canary-yellow color produced. This is undoubtedly due to the strictly aërobic condition of the straight tube containing a liquid, while in the straight tube with the solid agar or in the fermentation tube anaërobic conditions existed which apparently are necessary for this canary-yellow reaction. The change of color under aërobic conditions with and without dextrose was caused by the acid production. In the presence of sugar acids are produced which change neutral red to a magenta color. The production of acid was not tested except in the presence of dextrose, saccharose, lactose, and glycerin; but gas, which is an indication of acid production, was observed to form in the presence of levulose, galactose, and mannit.

TABLE XIII.—*Growth of various cultures of March 18, 1910, on agar, containing neutral red and dextrose, at 37° C.*

Culture.	1 day.	3 days.	5 days.
Check	Red	Red	Red.
Coconut 1	Red; gas; pink surface growth.	Lower three-fourths canary yellow; upper part pink; pink growth on surface; color somewhat bleached at top.	Practically all bleached to a canary yellow.
Coconut 2	Yellowish-green spots in agar; gas; pink growth.	Lower three-fourths canary yellow; pink growth on surface.	Same as before.
Coconut 3	do	do	Do.
Coconut 4	do	do	Do.
Coconut 5	do	do	Do.
Coconut 6	do	do	Do.
Bacillus coli	do	do	Do.

This experiment was repeated with another lot of neutral-red agar, believed to be the same as the first lot, with the exception of titrating three degrees higher on the Fuller scale. Whatever the cause may have been there was no change in the color of the medium from pink to canary yellow. A moderate amount of pinkish growth appeared on the surface, but otherwise there was no characteristic reaction. This medium was made up in each case with 1 per cent agar flour, beef bouillon made with distilled water, 2 per cent dextrose, and enough neutral red to make a bright pink.

In MacConkey's bile-salt agar (for full description see p. 83) consisting of peptone, sodium taurocholate, lactose, and neutral red, the canary-yellow color in the lower part of the medium was very striking.

According to Hunter,[1] Rosenberger,[2] and Moore and Revis,[3] the neutral-red reaction is characteristic of *Bacillus coli* and a few other organisms. This reaction is thus useful in separating this group of organisms from others. Moore and Revis have found that under certain conditions the canary-yellow reaction does not always result. In particular they found that in the presence of glucose the reaction seldom occurred. Lactose was considered to be the best sugar to use, and the result in MacConkey's bile-salt agar containing lactose seems to verify this. It is stated by these authors that the canary-yellow color is only transitory when resulting in glucose media.

For a further test of the constancy of this canary-yellow reaction experiments were made with agar media without sugar, with lactose, with dextrose, with saccharose, and with glycerin. The six coconut organisms and *Bacillus coli* were grown in these media in two different experiments. Table XIV shows the results of these experiments with *B. coli* and coconut No. 5.

---

1 Hunter, William. The Diagnosis of the Presence of Bacillus Coli Communis by Means of Neutral Red. British Medical Journal, Sept. 21, 1901, pp. 791–792.

2 Rosenberger, R. C. The Identification of the Colon Bacillus by Reactions Produced in Culture Media Containing Neutral Red. Philadelphia Medical Journal, vol. 9, Mar. 8, 1902, pp. 446–449.

3 Moore, Ernest W., and Revis, Cecil. The Neutral-Red Reaction for Bacillus Coli Communis. Journal of Pathology and Bacteriology, vol. 10, 1904–5, pp. 97–104.

TABLE XIV.—*Growth of Bacillus coli and coconut No. 5 on agar containing neutral red and various sugars, May 9–21, 1910, at 37° C.*

Culture and medium.	1 day.	2 days.	7 days.	12 days.
Bacillus coli:				
Without sugar.	Pink growth; liquid in V greenish fluorescent.	Excellent growth in each tube.	Both growth and agar entirely changed to a greenish-orange yellow.	Orange color throughout.
With lactose...	Pink growth; slight greenish color in V	.....do............	Growth on surface bright pink.	No sign of change of color to greenish; all red.
With dextrose.	Same as without sugar.	.....do............	.....do..............	All red.
With saccharose.	.....do..............	.....do............	Almost entirely greenish yellow.	Greenish-orange yellow throughout.
With glycerin..	.....do..............	.....do............	Growth on surface, pink; one-third of agar greenish yellow.	Pink growth; agar greenish yellow, fluorescent.
Coconut 5:				
Without sugar.	Pink growth; bright green fluorescence in V.	Same as before, only in each case the green extends to bottom of tubes.	Changed from pink to orange yellow with a green tinge.	Orange color throughout.
With lactose...	.....do..............	.....do............	Bright red; shows no sign of greenish yellow.	Bright red.
With dextrose.	.....do..............	.....do............	.....do..............	Do.
With saccharose.	.....do..............	.....do............	Greenish yellow in lower part of front.	Most of growth is pink; firm part of agar greenish yellow.
With glycerin.	.....do..............	.....do............	Bright red; shows no sign of greenish yellow.	No change in color.

In these experiments coconut No. 1 reduced the color in nearly every instance. The remainder were for the most part like *Bacillus coli* and coconut No. 5. In nearly every case the culture in medium without sugar changed to the greenish-fluorescent and then to an orange-yellow color. In the media with lactose, dextrose, and glycerin the same greenish-fluorescent reaction took place over a part of the medium and growth, and then a darker purplish-red color appeared. In the medium with saccharose there is the same apparently permanent change to orange yellow from the pink to greenish fluorescence as in the tubes with no sugar.

MACCONKEY'S BILE-SALT AGAR WITH NEUTRAL RED.

The sodium taurocholate and the lactose in this medium are said to have an inhibitive effect on nearly all but the intestinal bacteria. The addition of neutral red further aids in separating the species. The medium here used was made up according to the method in Eyre's Bacteriological Technique, page 169.[1]

[1] See also Grunbaum, A. S., and Hume, E. H., "Note on Media for Distinguishing Bacillus Coli, Bacillus Typhosus, and Related Species," in British Medical Journal, June 14, 1902, pp. 1473–1474.

TABLE XV.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli on MacConkey's bile-salt agar with neutral red in slant tubes, April 22 to May 2, 1910, at 37° C.*

Culture.	1 day.	3 days.	6 days.	10 days.	18 days.
1	Greenish fluorescent liquid in lower part of V; in upper part, pink suspension; no gas; good pink growth like that on ordinary agar.	No gas; upper part of agar dull pink; lower part greenish yellow; pale pink growth on slant.	Somewhat bleached.	Somewhat bleached throughout.	Same as on tenth day.
2	Gas; otherwise like culture 1.	Gas; color like culture 1, except for bright pink growth on slant.	Same as on third day.	Bright pink growth on surface; portion of agar is greenish yellow.	Bright pink throughout.
3	No gas; otherwise like culture 2.	do	do	Same as culture 2, only more greenish yellow.	Only a tinge of greenish yellow.
4	do	No gas; growth and color like culture 2.	do	Bright pink throughout.	Bright pink throughout.
5	Same as culture 2	Gas; like culture 2.	do	do	Do.
6	Same as culture 3	do	do	do	Do.
Bacillus coli.	do	do	do	do	Do.

TABLE XVI.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli on MacConkey's bile-salt agar with neutral red on plates, April 26 to May 2, 1910, at 37° C.*

Culture.	2 days.	4 days.	6 days.	14 days.
1	Numerous fairly large white colonies.	Round, wet-shining, semitransparent, slightly pinkish colonies; agar translucent.	Same as on fourth day.	Same as on sixth day.
2	Many tiny submerged bright pink colonies; surface colonies small, round, white or with pinkish tinge.	Numerous tiny submerged pink colonies; moderate number of surface white or pinkish colonies; agar dull pink, opaque.	do	Pink surface colonies; bright pink submerged colonies; agar semiopaque.
3	Few colonies with peculiar tiny projections.	Numerous tiny submerged pink colonies; moderate number of surface white or pinkish colonies; agar translucent.	do	Same as culture 2, only agar is translucent.
4	Few colonies; not at all characteristic.	Same as culture 3	do	Do.
5	Same as culture 2	Same as culture 2	do	Like culture 2.
6	Few colonies; not at all characteristic.	Like culture 5, only agar is translucent.	do	Pink surface colonies bright pink submerged colonies; agar translucent.
Bacillus coli.	Like culture 2	Like culture 5	do	Like culture 2.

From these experiments it will be seen that the organism in question grew very well on this medium, equally well with the *Bacillus coli* used. There was a little variation in the plates, but all the tubes were practically alike with the exception of No. 1. It will be noted that the greenish-yellow fluorescence was only a transitory character, and that subsequent to it a bright pink or slightly purplish-pink semiopaque color was produced quite in contrast to the semitransparent orange-red of the check tubes. This reaction appears to be similar to that already discussed in the foregoing pages.

TEST 1 OF D. RIVAS.[1]

One-fourth c. c. of a 48-hour culture in neutral dextrose bouillon was rapidly boiled in about 5 c. c. of a 10 per cent solution of NaOH. Tests made with both the coconut organism and *Bacillus coli* gave the typical clear lemon-yellow color reaction of this test.

The color in this reaction is discharged by acid and restored by alkali. This reaction depends upon the biological action of the bacteria upon the sugar.

This experiment was also tried using beef bouillon +14 instead of neutral, with the same results. Cultures in 1 per cent peptone with 2 per cent dextrose, titrating +3 likewise gave the same lemon-yellow reaction.

TEST 3 OF D. RIVAS.

According to Dr. Rivas,[1] *Bacillus coli* does not exhaust all the sugar from a medium, at least if there is any large amount. On this ground he would separate this organism from closely allied ones which he would place in a so-called saccharolytic group, i. e., those capable of exhausting all the sugar. So incomplete is the exhaustion of sugar by *Bacillus coli* that it is inadvisable to use it for the purpose of freeing beef bouillon from the small amount of muscle sugar it may contain. *Bacillus cloacæ* is said to be much preferable. At least a partial explanation of this condition is that *Bacillus coli* produces so much acid in the presence of sugar that it prevents the extensive growth that would otherwise take place.

For the purpose of identifying the coconut organism with *Bacillus coli* tests were made of cultures in sugar solutions to ascertain the relative amount of sugar used in the growth of the organisms.

Two methods were used for determining the amount of sugar remaining in the cultures after a certain amount of growth. Fehling's solution was diluted with an equal amount of water and divided among a number of small test tubes, 1 cubic centimeter being placed in each. To these the cultures were added in increasing amounts, beginning with one, two, three, etc., drops up to 1 cubic centimeter, and the mixture was then boiled. In the other experiments a less accurate method was used. Fehling's solution was added directly to each 10 c. c. of the culture tubes. Amounts from 2 to 3 c. c. were added at a time and then boiled to bring about the reduction. Fehling's was added only until the light orange-red color of the heated solution began to change to a greenish tinge.

(1) Cultures of February 26 in medium 4192, tested after 55 days in Dunham's solution with 2 per cent dextrose. The average of six

[1] Rivas, D. Contribution to the Differentiation of Bacillus Coli Communis from Allied Species in Drinking Water. Journal of Medical Research, vol. 18, 1908, pp. 81–91.

tests with check solutions resulted in four drops of the sugar solution being sufficient to reduce completely 1 c. c. of Fehling's solution.

The cultures gave results as follows:

No. 2: 2 drops reduced 1 c. c. of Fehling's; that is, twice the amount of sugar. (This must be incorrect.)

No. 5: 4 drops reduced 1 c. c. of Fehling's; that is, no measurable amount of sugar was consumed.

No. 6: 5 drops reduced 1 c. c. of Fehling's; that is, one-fifth the amount of sugar.

Bacillus coli: 6 drops reduced 1 c. c. of Fehling's; that is, one-third the amount of sugar.

No. 3: 5 drops reduced 1 c. c. of Fehling's; that is, one-fifth the amount of sugar.

No. 4: 5 drops reduced 1 c. c. of Fehling's; that is, one-fifth the amount of sugar.

(2) Cultures in medium 4268 tested after five days in Dunham's solution with 1 per cent dextrose, incubated at 37° C.

Bacillus coli: 10 c. c. reduced 16.5 c.c. Fehling's = one-fifth of the total amount.

Bacillus coli a: 10 c. c. reduced 18 c. c. Fehling's = one-tenth of the total amount.

No. 5: 10 c. c. reduced 17 c. c. Fehling's = three-twentieths of the total amount.

No. 5 a: 10 c. c. reduced 18 c. c. Fehling's = one-tenth of the total amount.

According to calculation the check tube of 10 c. c. with 1 per cent dextrose requires 20 c. c. of Fehling's to reduce it.

(3) Cultures similar to those in experiment 2 tested after 47 days.

These cultures were dried down to less than one-half their original amount. As only a portion of each tube was tested the cultures were diluted to their original amount and well shaken up before using. It was found by repeated experiments with tubes of *Bacillus coli* and coconut No. 5 that 1 c. c. of the culture solution just completely reduced 1 c. c. of Fehling's solution. As 10 c. c. of Fehling's is supposed to reduce 0.05 grams of dextrose, 1 c. c. must reduce 0.005 grams, and as the amount of dextrose used was 0.01 grams to the cubic centimeter, one-half the original amount had been consumed by the bacteria.

(4) Cultures in medium 4193, 1 per cent peptone and 2 per cent dextrose, tested after 12 days.

Ten c. c. of culture No. 5 in this solution were able to reduce only 30 c. c. Fehling's, thus showing that about one-fourth of the sugar had been used.

It required 40 c. c. of Fehling's solution to be completely reduced by the 10 c. c. of the check culture solution.

(5) Cultures of February 26 in medium 4192, tested after 23 days. (See experiment 1.) The number of cubic centimeters of Fehling's solution reduced by 10 centimeters of culture was as follows:

No. 5, 38; *Bacillus coli*, 20; No. 1, 15; No. 3, 30; No. 6, 32; No. 4, 38; check, 43; check, 43.

(6) Cultures in medium 4229, neutral beef bouillon plus 1 per cent dextrose, after 48 hours at 37° C. The number of cubic centimeters of Fehling's solution reduced by 10 centimeters of culture was as follows:

No. 6, 10; No. 1 a, 10; No. 5, 10; No. 1, 10; No. 4 a, 11; No. 4, 5; No. 3, 10; *Bacillus coli*, 10; check 2, 20; check 5, 10.

The results in this experiment indicate that on an average one-half of the sugar was exhausted in 48 hours.

The experiments may be summarized as follows:

Experiment 1: In 2 per cent dextrose after 55 days.
Bacillus coli used one-third of the amount of sugar.
Coconut used one-fifth of the amount of sugar.
Experiment 2: In 1 per cent dextrose after 5 days.
Bacillus coli used two-twentieths to four-twentieths of the amount of sugar.
Coconut used two-twentieths to three-twentieths of the amount of sugar.
Experiment 3: In 1 per cent dextrose after 47 days at 37° C.
Bacillus coli used one-half of the amount of sugar.
Coconut used one-half of the amount of sugar.
Experiment 4: In 2 per cent dextrose after 12 days.
Bacillus coli not tested.
Coconut (No. 5) used one-fourth of the amount of sugar.
Experiment 5: In 2 per cent dextrose after 23 days.
Bacillus coli used one-half of the amount of sugar.
Coconut used one-twentieth to one-third of the amount of sugar.
Experiment 6: In 1 per cent dextrose after 48 hours at 37° C.
Bacillus coli used one-half of the amount of sugar.
Coconut used one-half to three-fourths of the amount of sugar.

In these experiments the amounts given for coconut are the average of the coconut organism series 1 to 6. The results indicate that from small quantities up to one-half [1] the amount of sugar in a 1 per cent or 2 per cent solution of dextrose is broken up by the organism. In experiment 6 the limit of coconut is given as three-fourths. This unusual amount may be due to error in the test, for it is difficult, even with the utmost care, to ascertain the exact end of the reduction in each case. In general, it seems safe to assume that any error lies on the side of reckoning too much sugar used rather than too little. It is a very easy matter to allow a little of the blue Fehling to stand unnoticed in the intense orange-red of the reduced solution. In these experiments, however, it is shown that *Bacillus coli* and the coconut organisms behave much alike in their relation to the sugar content of the medium.

[1] Scruel, M. Contribution à l'Étude de la Fermentation du Bacille Commun de l'Intestin." Archives Médicales Belges, ser. 4, vol. 1, 1893, pp. 9–33, 83–107.

M. Scruel records, for the amount of sugar consumed, the following: 1 day, 0.92 out of 3; 2 days, 1.22 out of 3; 3 days, 1.25 out of 3; 6 days, 1.28 out of 3. And another time: 1 day, 0.50 out of 2; 2 days, 0.78 out of 2; 3 days, 0.81 out of 2; 4 days, 0.88 out of 2.

GROWTH ON ENDO'S FUCHSIN AGAR.

Endo's method has been particularly discussed by Herford [1] and Ruata.[2] By the latter it has been stated that one difficulty with the method is the instability of the medium, due to the looseness of the combination of fuchsin with the sodium sulphite and the inconstancy of the color reaction. Notwithstanding this objection, the writer believes that the variation of the medium will be the same for *Bacillus coli* as for the coconut organism, so that the behavior of the organisms on this medium can be compared regardless of any such difficulty.

The method of making Endo's fuchsin agar as given by Ruata, is as follows:

Half a kilogram of powdered meat, 1 liter of water, 10 grams of peptone, 5 grams of sodium chlorid, and 30 grams of agar are boiled together; the mixture filtered and neutralized. Then 10 c. c. of a 10 per cent solution of sodium carbonate are added in order to render the fluid alkaline. Finally, 10 grams of lactose and 5 c. c. of an alcoholic solution of fuchsin are added. The medium assumes a deep-red color which disappears on the addition of 25 c. c. of a 10 per cent solution of sodium sulphite. The medium is then poured into tubes, each containing 15 c. c., and is sterilized by steam. In order to obtain good results all the constituents of this formula must be obtained pure, the solution of sodium sulphite must be kept well stoppered, and the solution of fuchsin must be filtered before using and must be kept in a dark place. When using this medium the agar, melted and cooled to 40° C., after inoculation is poured into sterilized petri dishes where it is allowed to solidify. These dishes are kept at 37° C., and after 15 hours colonies of the colon bacillus may be seen. After 24 hours these colonies become completely red and assume the greenish iridescence characteristic of fuchsin. In contrast to this reaction on the part of the colon bacillus, the typhoid bacillus remains colorless.

Ruata states that in his experiments both the bacillus of typhoid fever and *Bacillus coli* either turn the medium red or do not color it, according to the variety of the germ and the particular source in each case, as well as according to the nutrient medium in which they have been cultivated, the age of the cultures, the quantity of the material used for infection, etc.

[1] Herford, Max. Das Wachstum der zwischen Bacterium coli und Bacillus typhi stehenden Spaltpilze auf dem Endoschen Fuchsinagar. Arbeiten aus dem Kaiserlichen Gesundheitsamte, vol. 24, 1906, pp. 62–67.

[2] Ruata, Guido. Il Metodo di Endo per la Differenzlczione del Bacillo di Eberth del Bacillo del Colon. Reforma Medica, vol. 20, Apr. 27, 1904, pp. 449–453. Reviewed in the New York and the Philadelphia Medical Journal, July 16, 1904, p. 126.

TABLE XVII.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli on plates of Endo's medium (made by Ruata's method), April 21 to May 4, 1910, at 37° C.*

Culture.	1 day.	2 days.	4 days.	5 days.	7 days.	9 days.	13 days.
1	Fairly thickly sown with colonies; white to iridescent.	White colonies; no color, or sign of iridescence.	No sign of pink.	Still uncolored.	Still uncolored.	No color..	....
2	Same as culture 1.	Small area still white; rest bright pink.	Half of plate with white colonies; other half with pink.	Two-thirds of plate brightly colored.	Less than one-sixth pink.	...do.......	....
3	White colonies without iridescence.	Almost all of many colonies are bright pink. The light-colored ones are iridescent; colonies are smooth, wet shining.	Bright red colonies throughout; same as B. coli.	Same as before.	Same as before.	Bright red all over.	At 45° C. remains same.
4	.....do.........	Part of plate bright pink.	Small area on one side bright red.	.....do.......	No signs of pink.	No color..	....
5	Same as culture 1, with a slight pink tinge to surface of agar on one side.	Bright pink over two-thirds of plate.	About one-half bright red.	Bright red color over one-fourth of plate.	One-sixth of plate still pink.	...do.......	....
6	White colonies without iridescence.	Portion of colonies are bright pink, some with denser centers than margins.	Small area on one side bright red.	No pink colonies whatever; only a pinkish caste remaining in a few.	No sign of pink.	...do.......	....
Bacillus coli.	Well sown. A decided pink color throughout plate. Colonies pink in direct light; iridescent in reflected light.	All colonies and all of agar bright red; some colonies zonate.	Red throughout.	Bright red..	Remains bright red all over.	Bright red all over.	At 45° C. remains same.

From this first experiment it is seen that the reaction is not complete in all cases, and, moreover, it is not permanent in all cases In *Bacillus coli* and one of the coconut cultures (No. 3), which were placed in the thermostat at 45° C. after 9 days, the color reaction 13 days after inoculation was complete and apparently permanent. In other tubes portions of the plates became red and then bleached out. One culture (No. 1) failed to show the reaction.

TABLE XVIII.—*Growth of cultures Nos. 1 to 6 and Bacillus coli on plates of Endo's medium (Ruata's formula), April 26 to May 6, 1910, at 37° C.*

Culture.	2 days.	4 days.	6 days.	10 days.
Coconut 1..	Numerous white colonies.	Numerous white colonies.	Numerous white colonies.	No color.
Coconut 2..	Numerous round white colonies.	About one-fifth of colonies are pink.	Same as before........	Only a trace of pink.
Coconut 3..	.....do.................	Numerous round white colonies.	Many pink colonies about circumference; center white.	Do.
Coconut 4..	.....do.................	Some of colonies are iridescent.	Same as before........	Do.
Coconut 5..	.....do.................	Colonies same form; all bright pink in color.	.....do.................	Do.
Coconut 6..	.....do.................	Some of colonies are iridescent and some pink.	About one-eighth of the plate is pink.	Do.
B. coli.....	Numerous round white and some pink colonies.	Colonies all same form; all bright pink in color.	About one-fifth of the colonies are bright pink.	Bright red all over.

Slant-tube cultures on Endo's medium, May 4 to 11, 1910, at 37° C.

Two days: *Bacillus coli* and *Bacillus coli* a were bright pink. Some of the others showed a tinge of color but nothing more, although the growth was good.

Three days: Nos. 1 and 1 a are slightly pink. *Bacillus coli* and *Bacillus coli* a are bright red throughout the medium. All others show a bright pink surface growth, but the bottom of these tubes is colorless.

Five days: Nos. 1 and 1 a are slightly pink. All the others are bright red throughout. *Bacillus coli* and *Bacillus coli* a are a trifle brighter than the others. The growth is good in all cases; pink, smooth, and wet shining.

Seven days: No change. All but 1 and 1 a still retain their bright color.

From these experiments it may be seen that the reaction of the medium seems to be the same for the coconut cultures as for *Bacillus coli*. Luxuriant white colonies which appear in transmitted light like drops of water first develop on the medium. Then appears a slight pink color, as seen in direct light, or an iridescence passing from pink to green and blue, as seen in reflected light. Later the pink darkens to a deep red and the colonies appear opaque. There is no sign of the greenish metallic fluorescence characteristic of fuchsin and mentioned by Ruata as a part of the typical reaction with *Bacillus coli*. In an attempt to obtain this reaction on old cultures two plates were placed at 47° C. until they were completely dried down. The bright red deepened to a dark magenta, but in no case were there any signs of the fuchsin metallic luster.

In the original make-up of the medium the fuchsin is decolorized by the sodium sulphite. This action probably results in the formation of sodium sulphate and some colorless derivative of fuchsin. As a result of the growth of the organism some reducing agent is formed which removes the atom of oxygen from the sulphate and restores it to the fuchsin, thus yielding sodium sulphite and fuchsin if good growth takes place.

STODDART'S PLATE MEDIUM.

Stoddart's plate medium is used to distinguish *Bacillus coli* from *Bacillus typhosus.* Its value depends upon the fact that a nonmotile or slowly motile organism forms a thick nonspreading or slightly spreading growth on the surface, while a moderately or rapidly motile organism will quickly diffuse throughout the medium and over the surface. The efficiency of this medium seems to the writer to be impaired by the fact that not only *Bacillus typhosus* is rapidly motile but many forms of *Bacillus coli* are also. For the purpose of comparing the coconut cultures with those of *Bacillus coli,* however, the medium may well be of service.

The composition of the medium was that described in Novy's Bacteriology, page 492. It consisted of gelatin 5 per cent, peptone 1 per cent, agar 0.5 per cent, and NaCl 0.5 per cent. The method of using it was to pour into petri dishes and allow it to solidify. The organism to be tested was touched by means of an inoculating needle to the center of the surface of the medium. The Eberth bacilli are said to spread over the entire surface of the plate exposed at 35° C. for 18 hours and to form a transparent, scarcely visible growth. The nonmotile colon bacilli will form a small white colony on the surface without any diffusion. The motile colon bacilli will diffuse, but unlike the Eberth bacilli the growth will be opaque and easily visible.

Stoddart's plates, March 18, 1910, at 22° C.

After 18 hours:

Bacillus coli, and *Bacillus coli* a: Semiopaque growth over four-fifths of the plate.

Coconut 5, 5 a, 3, 3 a: Entirely overgrown with semiopaque growth.

Coconut 2, 2 a, 6, and 6 a: Same as *B. coli.*

Coconut 1, 1 a, 4, and 4 a: Nine-tenths overgrown; semiopaque growth.

The growth in all of these plates was very rapid, semiopaque, and wet shining. There was practically no difference between *Bacillus coli* and the coconut cultures. Evidently the strain of *Bacillus coli* here used and the coconut organism are rapidly motile.

HISS'S TUBE MEDIUM.

Dr. P. H. Hiss has used for differentiating the typhoid bacillus and the colon bacillus a certain "tube medium" and another "plate medium." Only the tube medium [1] has been tried by the writer. It consists of dextrose 1 per cent, beef extract 0.5 per cent, gelatin 8 per cent, agar 0.5 per cent, NaCl 0.5 per cent. Ordinary stab cultures are made. The colon bacilli give rise to gas bubbles, whereas the Eberth bacillus does not.

[1] Hiss's tube medium. Novy, Frederick G. Laboratory Work in Bacteriology, p. 494. Also Studies from the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York, vol. 5, 1897–98, pt. 2; and Journal of Medical Research, vol. 8, 1902, pp. 148–167.

Hiss's tubes.

April 14, 1910, at 22° C. One day: All the tubes, both *Bacillus coli* and the coconut, show abundant gas bubbles which are well distributed throughout the medium. Two days: Same.

March 18, 1910, at 37° C. One day: In all the tubes the medium is clouded throughout, and many gas bubbles are scattered throughout.

In these tubes, as on the Stoddart plates, *Bacillus coli* and the coconut organism behaved alike and showed active motility.

GROWTH IN STERILE MILK.

Cultures of the coconut organism grown in sterile milk at room temperature coagulated the milk in from three to four days. It became a solid homogeneous mass and little or no whey was extruded. No subsequent digestion of the curd took place. Incubated at 37° C., the organism usually caused coagulation in two or three days; but some variability was shown.

GROWTH IN LITMUS MILK.

Cultures grown in litmus milk (lavender blue) usually changed the color of the medium within 24 hours to a dark lavender red, and within 48 hours it became lighter. At the end of two or three weeks the lower part of the culture became bleached. The milk itself gradually coagulated, as in the case of the sterile milk cultures, and usually no whey was extruded. (For further discussion of growth in plain and litmus milk see pp. 94–96.)

Products of Growth of the Organism.

PRODUCTION OF INDOL AND PHENOL.

Cultures of the coconut organism were made in Dunham's solution, which quickly clouded. After six days sulphuric acid was added, which, even after standing, failed to show any reaction. The addition of sodium nitrite to this, however, turned all of the tubes strongly pink in color, showing the presence of indol. This experiment was repeated with cultures of eight days' growth and a light pink resulted from the test. A repetition of this experiment, using a five days' growth and comparing with *Bacillus coli,* gave a light pink identical in each case. It is evident that this organism develops indol much the same as *Bacillus coli,* but whether in the end it develops as much is uncertain.

Cultures of the coconut organism, together with four strains of *Bacillus coli,* were grown in Dunham's solution. The tubes were incubated at 37° C. and tested at the end of 10 days. The results showed that all four of the *Bacillus coli* strains produced an equal amount of indol, and that each of the coconut organisms produced

nearly the same amount, respectively, except coconut No. 3, which showed as much as *Bacillus coli.*

Other cultures of the organism were made in ordinary bouillon, and an attempt was made to separate indol and phenol, if present, by distillation. No results were obtained, either by the sulphuric-acid and sodium-nitrite test for indol or by the Millon's reagent and the ferric-chlorid test for phenol. These experiments were repeated several times, and the same results were obtained. It would seem, therefore, that a small amount of indol may be produced, but no phenol.

PRODUCTION OF HYDROGEN SULPHID.

Cultures of the coconut organism made in an iron-peptone solution had in a week's time a slightly or wholly blackened precipitate, and the solution was either inclined to be a greenish black or was intensely green and black, thus indicating the production of hydrogen sulphid. Lead acetate paper used for testing the solution became discolored, also indicating the presence of hydrogen sulphid.

Cultures were also made directly in a lead acetate solution with peptone and showed a good growth. The precipitate in all of the culture tubes was black, indicating the production of H_2S, while in the check tube the precipitate was white. These cultures were also tested with lead acetate paper, which showed the brown-black discoloration typical of H_2S.

PRODUCTION OF AMMONIA.

A 250-c. c. flask containing 100 c. c. of beef bouillon + 15, was inoculated with the organism and incubated for 18 days. The culture was then distilled with the addition of 2 grams of calcined magnesia, and to 50 c. c. of the distillate was added 1 c. c. Nessler's solution. A bright orange-yellow color was produced. Checks were made by distilling over uninoculated bouillon which gave a gray-black color with Nessler's solution and by the use of solutions of ammonium hydrate, 1 to 1,000, 1 to 5,000, 1 to 4,000, and 1 to 3,333⅓. All of the solutions containing ammonia gave an orange color on the addition of Nessler's solution. The color of the reaction of the culture most nearly corresponded to the check solution containing 1 to 4,000 of ammonia.

Cultures were made in Fischer's solution, plus 1 per cent dextrose, plus 1 per cent KNO_3. The solution contained dipotassium phosphate, magnesium sulphite, and calcium chlorid. The growth of the organism after three weeks was fair. The culture was distilled over and tested for ammonia. The distillate showed the presence of a very small quantity of ammonia, about 1 to 80,000. Unfortunately, however, a check flask on being distilled over also showed about the same

amount present. The only conclusion is that there was some impurity in the chemical used. It is probable that ammonia would be produced only in the presence of some product as peptone or such as might be in beef bouillon. In a solution containing merely peptone plus NaCl (Dunham's solution) check tube titrated +9, and cultures grown 11 days were only +5, indicating a slight alkali production.

ENZYMES IN MILK.

In the coagulation of milk by the coconut organism the question arises whether the reaction was due to the acid formed or to an enzyme produced. This question has been discussed by O'Hehir [1] and by Savage,[2] both of whom claim that there may be a small degree of enzymatic action as well as acid coagulation.

Cultures of both the coconut organism and *Bacillus coli* were made in sterile litmus milk tubes. After incubation for nine days, when a good coagulation had taken place in all the tubes, ammonia was added to the tubes in quantity more than sufficient to neutralize the acid in the cultures. Practically complete dissolution of the curd quickly took place. The only residue left might be attributed to the small amount of fat in the tubes, as it had not been completely removed in the preparation of the medium. This experiment would indicate the coagulation to be entirely an acid one.

Attempts were made to free milk completely from its fat by repeated boiling and subsequent skimming off of the film formed on the surface, but without success.

Dr. Erwin F. Smith suggested the addition of calcium carbonate to the milk to take up the acid formed by the growth of the organism. Accordingly, cultures were made in litmus milk and in plain sterile milk, both containing 10 per cent $CaCO_3$. Coagulation took place, and the tubes were subsequently treated with ammonia. Their behavior and appearance are shown in Tables XIX and XX.

[1] O'Hehir, C. Jocelyn. A Note on the Coagulation of Milk by Bacillus Coli Communis. Journal of Pathology and Bacteriology, vol. 11, 1906–7, pp. 405–407.

[2] Savage, W. G. The Coagulation of Milk by Bacillus Coli Communis. Journal of Pathology and Bacteriology, vol. 10, 1904–5, pp. 90–97.

TABLE XIX.—*Coconut cultures Nos. 1 to 6 and Bacillus coli in litmus milk with calcium carbonate, at 37° C, May 27 to June 6, 1910.*

Culture.	Untreated.		Ammonia added in excess.	
	4 days.	5 and 6 days.	Immediate effect.	After standing 3 days.
1 and 1 a...	Soft curd with one-fifth whey; lavender color.	Pale lavender and semisolid.	Does not appear to dissolve the curd.	
2 and 2 a...	Bleached except for thin pink layer at top; firm curd.	Same as on fourth day.	Apparently all dissolved.	
3 and 3 a...	Lavender; semisolid..	.....do................	Apparently no dissolving.	No residue remaining.
4 and 4 a...	.....do................	....	....	
5 and 5 a...	Pink; firm curd.......	Lavender; firm curd..	No dissolving discernible.	Do.
6 and 6 a...	Same as cultures 3 and 3 a.	Pale lavender; semisolid.	No dissolving action...	Do.
Bacillus coli and Bacillus coli a.	Half bleached, rest pink; curd firm; some whey.	Same as on fourth day.	.....do................	Do.

TABLE XX.—*Coconut cultures Nos. 1 to 6 and Bacillus coli in plain sterile milk with calcium carbonate, at 37° C., May 27 to June 6, 1910.*

Culture.	Untreated.		Ammonia added in excess.	
	4 days.	5 and 6 days.	Immediate effect.	After standing 3 days.
1 b and 1 c.....	A soft curd; about one-fifth whey.	Part firm curd and rest whey; less whey in 1 c than in 1 b.	Apparently no effect in dissolving.	Curd appears completely dissolved.
2 b and 2 c.....	.....do........	2 b, fairly firm curd; 2 c, almost solid; moderate amount of whey in both.	.....do................	2 b, appears completely dissolved; 2 c, shows a translucent gelatinous portion.
3 b and 3 c.....	.....do........	3 b, almost solid; 3 c, semisolid. Moderate amount of whey in both.	.....do................	Both show a translucent gelatinous portion.
4 b and 4 c.....	.....do........	Fairly firm curd; moderate amount of whey.	.....do................	Appear completely dissolved.
5 b and 5 c.....	.....do........	Solid curd with small amount of whey.	.....do................	Thick gelatinous, translucent mass.
6 b and 6 c.....	.....do........	Soft; slightly acid to neutral litmus paper.	.....do................	6 b, shows a thin gelatinous, translucent layer at bottom.
Bacillus coli b and c.	.....do........	Solid curds with small amount of whey.	.....do................	Both have a translucent gelatinous portion.

From these experiments it will be seen in the first place that the $CaCO_3$ did not entirely prevent the acid from producing an effect on the litmus, i. e., reddening it. Consequently, the curd produced may have been the result of this acid. When the ammonia was added it appeared to have no determinate immediate effect. The curd was finally broken up by means of a glass rod and thoroughly mixed with the ammonia. The broken fragments of curd showed no sign of immediate disappearance; but after the tubes were allowed to stand for three days there were no signs whatever of the curd in certain tubes. These cultures were diluted and strained through a

filter paper without leaving the slightest trace of residue other than what was apparently the $CaCO_3$. In other tubes, on the contrary, there still persisted, not a distinct curd, but a residue, gelatinous in appearance—a small amount in several tubes, but a large amount in others. The nature of this mass was not ascertained. It was by no means similar to the cheesy curd of acid formation; yet it appeared to represent a coagulation of some sort. These experiments seem to justify the conclusion that the major part of coagulation is caused by the acid formation, but that a small amount of coagulation may also be due to an enzymatic action.

PRODUCTION OF ALCOHOLS, ALDEHYDES, AND ACETONE.

In testing for alcohols, aldehydes, and acetone 500 c. c. of a medium consisting of peptone and dextrose, to which 10 c. c. of calcium carbonate was added, was inoculated in a liter flask and incubated at 37° C. In two days the organisms had produced a large amount of gas which, however, had completely disappeared in seven days. Then a cubic centimeter of paraffin was thrown into the cultivation and the flask was connected with a condenser for distillation. The paraffin was for the purpose of forming a thin layer over the surface of the fluid to prevent frothing up and running over into the condenser. The distillate obtained was about 300 c. c., which was then tested for alcohols, aldehydes, and acetones. It was divided into four portions and tested. To one portion was added Lugol's iodin (iodin, 1 gram; iodid of potassium, 3 grams; distilled water, 300 c. c.), then a little NaOH solution to the liquid, which was stirred with a glass rod. Abundant pale-yellow crystalline precipitate was formed, which indicated the presence of iodoform, which was very evident also from the odor. This reaction indicated that either alcohol, aldehyde, or acetone was present, and further tests were made for their identification.

To a portion of the solution enough ammonia was added to make the solution strongly alkaline and then gradually a solution of iodin in ammonium iodid was added. A black precipitate formed,[1] but no other change took place, thus indicating the absence of acetone.

In order to determine the presence of alcohol, 1 cubic centimeter of molybdic acid was gently warmed in 10 c. c. of strong H_2SO_4, and then a few drops of the distillate were added and warmed in a porcelain dish for a few moments. A bright Prussian-blue color resulted, indicating the presence of an alcohol.

In order to test for the presence of an aldehyde, a solution of phenol in excess of sulphuric acid was made up and to it was added a small amount of the distillate. The absence of any resulting dark-red color

[1] M. Seruel says (Archives Médicales Belges, ser. 4, vol. 1, 1893, pp. 9-33) that acetone is reported as occurring in *Bacillus coli* cultures.

on warming the mixture indicated the absence of any aldehydes. From this distillate the presence of alcohol only was thus demonstrated.

PRODUCTION OF VOLATILE AND FIXED ACIDS.

The residue from the distillate for alcohol was used for detection of acid production. The flask was disconnected from the condenser and the calcium carbonate filtered from the residue. Ten cubic centimeters of concentrated hydrochloric acid were then added to this filtrate and mixed well. The calcium remaining in the filtrate was precipitated by adding sodium carbonate solution to alkalinity. The mixture was thoroughly boiled to insure complete precipitation. It was then filtered and 20 c. c. of 25 per cent sulphuric acid were added to the filtrate for the purpose of liberating the volatile acids; finally the filtrate was distilled as completely as possible. (This distillate will contain the volatile acid, if one be present.) The solution was first saturated with baryta water to alkalinity and then evaporated to dryness. To this 20 c. c. of absolute alcohol were added, and it was allowed to stand with frequent stirring for about three hours, when it was filtered and washed with alcohol. This last filtrate should contain barium propionate and barium butyrate, if present. The filtrate was evaporated to dryness; the residue was dissolved in 150 c. c. of water and saturated with calcium chlorid. It was then distilled and the distillate tested for butyric acid. Three cubic centimeters of alcohol and four drops of concentrated sulphuric acid were added to a part of the solution, but there was no resultant pineapple odor to indicate the presence of butyric acid. The propionic acid was not given a special test.

The residue from the alcohol washing described in the previous paragraph was treated for barium acetate and barium formate. It was first dried, and the residue dissolved in the filter in hot water, and the resultant solution was divided into four portions. To one portion was added ferric-chlorid solution, and the absence of any brown color gave negative results for the presence of acetic or formic acid. To another portion silver-nitrate solution was added and then one drop of ammonium water, and the solution was boiled. A black precipitate resulted from this, which indicated the presence of formic acid. To another portion a few drops of mercuric-chlorid solution were added and heated to 70° C. There was, however, no distinct precipitate of mercurous chlorid nor a formation of a metallic mirror. Thus, the tests suggested the presence of formic acid without absolutely proving it, while they indicated the absence of acetic acid.

The residue remaining from the distillation of the mixture after the addition of sulphuric acid was tested for the fixed acids. It was evaporated to a syrup and then extracted with ether by agitation in

the separatory funnel. The ethereal extract was evaporated to a syrup and a small residue was left, thus suggesting the presence of either lactic, oxalic, or succinic acid. To the extract was added and thoroughly mixed 100 c. c. of water. Then an excess of zinc oxid was added, and the mixture was heated nearly to boiling and filtered. To 6 c. c. of the filtrate were added 4 c. c. of concentrated sulphuric acid, and the whole was warmed to 75° C. The absence of any crimson color indicated the absence of glycocholic, taurocholic, or cholic acid.

To another portion of the filtrate was added Lugol's iodin, and the absence of any blue color here also indicated the absence of any chloric acid. Another portion of the filtrate was acidified with hydrochloric acid. Ammonia was added in slight excess, and the excess then boiled off. A solution of cobalt nitrate was added, and absence of any lactic acid was indicated by the lack of a violet color.

Another portion of the filtrate was evaporated to dryness and then dissolved in 10 c. c. of hot water and allowed to crystallize, but there resulted only a yellowish amorphous mass which indicated the absence of any crystals of zinc lactate.

The residue left from the filtering after the addition of zinc oxid was dissolved in hydrochloric acid on the filter, and then a portion tested for oxalic acid as follows:

It was neutralized with ammonia until faintly alkaline, and then a solution of calcium chlorid was added. There was no resultant white precipitate of calcium oxalate, which indicated the absence of oxalic acid.

Another portion of this filtered residue was neutralized with ammonia, and the excess boiled off. To a portion of this was added ferric-chlorid solution on a glass rod. A distinct red-brown coloration showed the presence of succinic acid. The absence of buff coloration indicated the lack of any benzoic or hippuric acid in the solution; the absence of a violet coloration indicated the lack of any salicylic acid; and the absence of an inky coloration indicated the lack of tannic or gallic acid in the solution.

This last series of tests for oxalic, succinic, benzoic, hippuric, salicylic, tannic, and lactic acids was repeated, and the same results obtained.

So far as this analysis shows, only succinic acid was certainly demonstrated to be present, and possibly formic. It has been shown by other investigators [1] that in the case of *Bacillus coli*, acetic, formic,

[1] M. Scruel reported (Archives Médicales Belges, ser. 4, vol. 1, 1893, pp. 9–33) finding lactic, acetic, and formic acids.

Leo. F. Rettger (Studies from the Rockefeller Institute for Medical Research, vol. 1, 1904, pp. 284–293) reports finding in egg-meat cultures of *Bacillus coli*, indol, skatol, phenols, aromatic oxyacids, skatolcarbonic acid, leucin, tyrosin, trytophan, hydrogen disulphid, mercaptan, albumoses, and peptones.

Arthur Harden (Journal of Hygiene, vol. 5, 1905, pp. 488–493) states that he found lactic acid, acetic acid, and a small amount of succinic acid present in glucose cultures of *Bacillus coli*.

and lactic acids were present, and succinic in small amount. The coconut organism is so similar to *Bacillus coli* in its cultural characteristics that it would be very surprising if it were not likewise similar in its chemical products. The foregoing single analysis is not sufficient to show that they are not the same in this respect, and it should be repeated.

Dr. Smith gave flasks of this organism (grown in river water containing Witte's peptone, Merck's dextrose, and calcium carbonate) to Dr. Carl L. Alsberg for quantitative chemical analysis, who reported as follows:

Received from Dr. Erwin F. Smith, February 23, 1910, one flask labeled "4101 February 4, 1910, Coconut from Agar, February 2, 5083, fr. 5 January 26."

The culture flask contained a white deposit, which on close inspection was seen not to be homogeneous, for in addition to the calcium carbonate put into the flasks before inoculation there were a few crystalline crusts, the total bulk of which was small. The precipitate was removed by filtration. The filtrate was acid and on warming some carbonic acid gas was liberated.

A part of the culture liquid filtered free from the calcium carbonate was acidified with sulphuric acid and exhausted with ether. The ether on evaporation left a mass of white crystals which after repeated recrystallization from hot water had a melting point of 183–4° C. uncorr. These crystals gave a very powerful pyrrol reaction (Neuberg). The aqueous solution was neutralized with ammonia and an excess of silver nitrate added. The resulting white silver salt was filtered off with suction and washed successively with water, alcohol, and ether. After drying in a desiccator 0.6655 gram was weighed into a crucible and ignited to constant weight. 0.4305 gram silver remained, or 65.12 per cent. The amount calculated for silver succinate is 64.70 per cent. On the basis of the silver content of the silver salt, the melting point of the free acid, and the pyrrol reaction, it is safe to say that this substance is undoubtedly succinic acid.

The mother liquor from which the succinic acid had been removed was subjected to distillation with steam. The distillate was quite acid. It was neutralized with ammonia, and silver nitrate added. The latter was immediately reduced to metallic silver, so that formic acid was probably present. The black silver precipitate was removed, and the clear filtrate concentrated in a desiccator. A crystalline crust, gray in color, formed in the course of a few days. This was removed, washed and dried, and, though obviously impure, its silver content was determined. 0.2305 gram yielded 0.1365 gram silver, or 59.23 per cent. As this corresponds neither to silver acetate (64.67 per cent Ag) nor to silver propionate (59.67 per cent Ag), and as the preparation was obviously impure, the determination was repeated.

Another culture was taken and after removal of the calcium carbonate distilled with the addition of a little sirupy phosphoric acid. The acids in the distillate were converted into the barium salts by evaporating on the water bath with an excess of barium carbonate. To the solution of the barium salts an amount of silver nitrate was added sufficient to combine with only a portion of the acid. On standing over night beautiful long white needles were formed. These were removed, washed and dried, and the silver content determined. 0.3925 gram yielded 0.2530 gram silver, or 64.46 per cent. Silver acetate contains 64.67 per cent. It is therefore evident that beside formic acid there can be present no other volatile acid but acetic.

The presence of formic acid was further verified by distilling a fresh portion of the culture liquid after it had been rendered faintly alkaline with sodium carbonate. Under these conditions any aldehyde which may have reduced the silver in the

preceding experiments, would distill over, while all the volatile acids would remain behind. The distillate failed to reduce ammoniacal silver solution, thus demonstrating the absence of volatile aldehydes. The distillate did, however, give a powerful iodoform test, showing the presence of alcohol.

The residue from the distillation was made acid with syrupy phosphoric acid and distilled again to drive over volatile acids. The presence of formic acid was verified in an aliquot part of the distillate by the reduction of mercuric chloride in the presence of sodium acetate. The calomel formed was weighed, so that the formic acid contained in one culture flask was estimated quantitatively with some degree of accuracy. The 700 c. c of the culture liquid contained 0.197 gram formic acid.

With ferric chloride the distillate gave a deep blood-red color characteristic of ferric acetate, a verification of the finding of acetic acid.

The crystalline crusts mentioned in the beginning of this paper seemed to consist mainly of calcium succinate. Oxalic acid could not be found. Lactic acid could not be found.

The culture liquid still reduced Fehling's solution powerfully. This was at first supposed to be due to the presence of unfermented glucose. However, the presence of formic acid was certainly responsible for a part if not all of this reduction. The culture liquid was not tested for glucose, so that the presence of glucose was not decided.

Summary: The organism forms much succinic acid and alcohol, as well as appreciable quantities of acetic and formic acid.

REDUCTION OF COLORS.

Cultures in litmus milk soon turned red and eventually usually bleached, at least in the lower part. The entire culture never lost its color, but frequently the lower one-half to two-thirds became reduced.

Cultures in litmus bouillon also reddened and either became almost entirely bleached or partially so.

Cultures in fermentation tubes containing beef bouillon and 1 per cent cane sugar and litmus became entirely bleached in the closed end, but unchanged in the open end of the tubes, both in the case of *Bacillus coli* and of the coconut organism. When the tubes are made up with a higher per cent of sugar, for instance, 3 or 5 per cent, the closed end of the tube becomes bleached on steaming and expulsion of the air from that end. According to Dr. Theobald Smith,[1] cultures of *Bacillus coli* grown in these tubes of litmus-sugar bouillon with the bleached closed ends cause the return of the litmus color. This reaction has not been tried by the writer.

A series of cultures was made in Dunham's solution (1 per cent peptone plus 0.5 per cent NaCl) and litmus; in Dunham's solution plus indigo carmine, and in Dunham's solution plus methylene blue, both with and without grape sugar. In the cultures with litmus, in one experiment, the color was reduced only in the tubes containing grape sugar. When cultures were grown in another lot of the

[1] Smith, Theobald. The Fermentation Tube. The Wilder Quarter-Century Book, 1893, p. 190.

Dunham's solution plus litmus with and without grape sugar, reduction either entire or partial took place.

In the Dunham's solution cultures containing indigo carmine there was no reduction either in the tubes with sugar or in those without.

In the Dunham's solution cultures containing methylene blue there was no reduction in color except in one tube containing the grape sugar.

From these experiments it would seem that a reducing agent is not always produced and does not affect all colors. As seen on other pages (pp. 69, 80, 115) cultures were made in neutral red and in rosolic acid. In these cases, however, the action was a complex one caused by the presence of an acid or an alkali, so that a clear reducing action could not be determined; there would seem to be one in neutral red but not in rosolic acid.

Growth on Miscellaneous Culture Media.

The media used in the following experiments are for the most part such as are commonly used in general cultural studies of a bacterial organism. In some cases they have little or no value in diagnostic work, but they serve as means to increase our knowledge of the life processes of the organism under investigation. In a few cases the media used were originally recommended by their authors as means of diagnosing or differentiating *Bacillus coli* from other organisms. For various reasons, under the writer's manipulations some of these tests have failed of their original purpose, but will here serve well as a means of comparison between the coconut organism and *Bacillus coli.*

NITROGEN-FREE MEDIA.

Cultures were made in a nitrogen-free nutrient medium plus various chemicals containing nitrogen to ascertain from which the organism could obtain its supply. Three salts of ammonium (tartrate, citrate, and lactate), asparagin, and sodium asparaginate were used. The nutrient medium was made up in the proportion of 1,000 c.c. triple distilled water, 5 grams of cane sugar, 2 grams of monopotassium phosphate, 0.1 gram of magnesium sulphate, and 0.5 gram of sodium chlorid.

TABLE XXI.—*Growth of various cultures in nitrogen-free media with various additions, at 22° C.*

EXPERIMENT 1, FEBRUARY 9 TO 25, 1910.

Medium and days of incubation.	Culture.						
	Coconut 1.	Coconut 2.	Coconut 3.	Coconut 4.	Coconut 5.	Coconut 6.	B. Coli.
Ammonium tartrate:							
1 day	Well clouded	Well clouded	Barely clouded	Barely clouded	Well clouded	Barely clouded	Barely clouded.
5 days	do	do	do	do	do	do	Do.
9 days	do	do	Practically clear	Practically clear	do	Practically clear	Practically clear.
15 days	do.[1]	do.[1]	do.[2]	do.[2]	do.[1]	do.[2]	Do.[2]
Ammonium citrate:							
1 day	do	do	Barely clouded	Barely clouded	do	Barely clouded	Barely clouded.
5 days	do	do	Well clouded [3]	Well clouded [3]	do	Well clouded [3]	Do.
9 days	do	do	do	do	do	do.[3]	Clear.
15 days	([4])	([5])	Partial film	Thick film [4]	([5])	([5])	Do.[6]
Ammonium lactate:							
1 day	Moderately clouded.	Moderately clouded.	Thinly clouded	Thinly clouded	Moderately clouded.	Thinly clouded	Thinly clouded.
5 days	Well clouded	Well clouded	Well clouded [7]	Well clouded [7]	Well clouded	Well clouded [7]	Well clouded.
9 days	do	do	Heavy film [8]	Heavy film [8]	do	Heavy film [8]	Do.
15 days	do	do	Slightly brown solution.	do.[8]	do	do.[8]	Do.
Asparagin:							
1 day	Moderately clouded.	Moderately clouded.	Thinly clouded	Thinly clouded	Moderately clouded.	Thinly clouded	Thinly clouded.
5 days	Well clouded	Well clouded	Well clouded [9]	Well clouded [9]	Well clouded	Well clouded [9]	Well clouded.
9 days	do.[10]	do.[10]	do.[11]	do.[11]	do.[10]	do.[11]	Do.[10]
15 days	([1])	([1])	([1])	([1])	([1])	([1])	([1])
Sodium asparaginate:							
1 day	Well clouded	Well clouded	Barely clouded	Barely clouded	Well clouded	Barely clouded	Barely clouded.
5 days	Moderately clouded.[11]	Moderately clouded.[11]	Moderately clouded.[9]	Thinly clouded [9]	Moderately clouded.[11]	Moderately clouded.[9]	Thinly clouded.[11]
9 days	do	do	Thinly clouded [9]	do.[9]	do	Thinly clouded [9]	Moderately clouded.
15 days	Well clouded [4]	Thinly clouded [12]	Well clouded [10]	do.[13]	Thinly clouded [12]	do.[12]	Well clouded.[10]

EXPERIMENT 2, FEBRUARY 21 TO MARCH 14, 1910.

Medium and days of incubation.	Culture.						
	Coconut 1.	Coconut 2.	Coconut 3.	Coconut 4.	Coconut 5.	Coconut 6.	B. Coli.
Ammonium tartrate:							
4 days	Moderately clouded.	Moderately clouded.	Barely clouded	Barely clouded	Moderately clouded.	Barely clouded	Barely clouded.
21 days	do	do	Clear [2]	Clear [2]	do	Clear [2]	(11)
Ammonium citrate:							
4 days	do	do	Moderately clouded.[11]	Moderately clouded.[11]	do	Moderately clouded.[11]	Barely clouded.
21 days	(14)	(14)	(15)	(15)	(14)	(15)	Clear.
Ammonium lactate:							
4 days	Moderately clouded.	Moderately clouded.	Moderately clouded.[11]	Moderately clouded.[11]	Moderately clouded.	Moderately clouded.[11]	Moderately clouded.
21 days	(14)	(14)	(16)	(16)	(14)	(16)	(17)
Asparagin:							
4 days	Well clouded [2]	Well clouded [2]	Well clouded [2]	Well clouded [2]	Well clouded [2]	Well clouded [2]	Well clouded.[2]
21 days	Moderately clouded.	Moderately clouded.	Moderately clouded.[11]	Moderately clouded.[18]	Moderately clouded.	Moderately clouded.[11]	Moderately clouded.
Sodium asparaginate:							
4 days	Well clouded [19]	Well clouded [19]	Well clouded [19]	Well clouded [19]	Well clouded [19]	Well clouded [19]	Well clouded.[19]
21 days	Moderately clouded.[20]	Moderately clouded.[20]	Moderately clouded.[21]	Moderately clouded.[21]	Moderately clouded.[22]	Moderately clouded.[21]	Moderately clouded.[21]

1 Good white precipitate.
2 Small precipitate.
3 With better film.
4 Heavy white precipitate.
5 Good brown precipitate.
6 Minute precipitate.
7 Thicker film.
8 Clearer than others.
9 Good film.
10 No film.
11 Thin film.
12 Heavy, slightly brown precipitate.
13 Good film; slightly brown precipitate.
14 White precipitate and solution.
15 Brown precipitate and solution; thin film; few crystals.
16 Brown precipitate and solution; thin film; many crystals.
17 Brown precipitate and solution.
18 Thin film; many crystals.
19 Moderate amount of white precipitate.
20 White precipitate; solution colorless.
21 Brown precipitate and solution; thin film; crystals.
22 Slightly brown precipitate and solution; thin film; crystals.

The two experiments show very much the same results, the only difference being a browning of the solution and precipitate of the scantily growing cultures in the second experiment. In these experiments cultures Nos. 1, 2, and 5 seem to be identical; and 3, 4, 6, and *Bacillus coli* identical with each other and different from 1, 2, and 5. This variation may not, however, be constant, and is certainly not of specific value. Considering these groups different, it would show the following improbable results: No. 3 (505 N) was inoculated into a tree producing a disease from which was isolated No. 2 (505 S) identical with it. No. 2 was inoculated into a tree and produced the disease and from it was isolated No. 1 (505 R), an organism differing slightly in the growth in the nitrogen compound. No. 1 was not tried to see if it has the same pathogenic properties as No. 2. Again, No. 6 (508 N), identical with No. 3, was inoculated into a tree and produced a disease from which was isolated No. 5 (508 S), identical with Nos. 1 and 2, but different from No. 4. Then No. 5 was inoculated into another tree, and from the resulting diseased tissue was isolated No. 4 (508 R), different from No. 5, but identical with No. 6. The assumption must be either that the organism is variable or that there are numerous organisms to be found in such places which are so nearly alike that they may be considered identical for practical purposes—that is, all have an identical disintegrating action on the plant tissues. Moreover, the chance in favor of there being separate forms is reduced to a minimum by the method of inoculation and isolation, every precaution being taken to avoid contamination.

FISCHER'S MINERAL SOLUTION WITH VARIOUS NUTRIENT SUBSTANCES.

For determining the source of nitrogen and carbon for the organism various compounds containing these substances were added to Fischer's mineral solution, which contained neither nitrogen nor carbon. The mineral solution consisted of dipotassium phosphate 1 gram, magnesium sulphate 0.2 gram, and calcium chlorid 0.1 gram, all dissolved in 1,000 c. c. of distilled water.

TABLE XXII.—*Experiment 1. Fischer's mineral solution with various additions. Inoculations made from fluid coconut cultures Nos. 1 to 6, February 8 to 18, 1910, at 22° C.*

Medium.	1 day.	3 days.	6 days.	11 days.	14 days.
Fischer's mineral solution (4110).	All equally and slightly clouded.	Same as 1 day.	Barely clouded; no precipitate.	Practically clear; no appreciable precipitate.	Same as 11 days.
Fischer's + dextrose (4113).	All equally and thinly clouded; not so good as in dextrose + KNO_3.	Thinly clouded.	Slightly clouded; minute precipitate.	1 thin; others clear; minute precipitate.	Do.
Fischer's + KNO_3 (4112).	All equally and slightly clouded.	Slightly clouded.	Barely clouded; no precipitate.	Clear; minute precipitate.	Do.

TABLE XXII.—*Experiment 1. Fischer's mineral solution with various additions. Inoculations made from fluid coconut cultures Nos. 1 to 6, February 3 to 18, 1910, at 22° C.*—Continued.

Medium.	1 day.	3 days.	6 days.	11 days.	14 days.
Fischer's + cane sugar (4115).	All equally and slightly clouded; not so good as in dextrose.	Slightly clouded.	Barely clouded; no precipitate.	Practically clear.	Same as 11 days.
Fischer's + peptone (4116).	All equally and thinly clouded.	Moderately clouded.	Moderately clouded; small precipitate.	Moderately clouded; moderate precipitate.	Do.
Fischer's + peptone + dextrose (4118).	Moderately clouded; some gas; best growth.	Same as 1 day.	Nos. 2 and 5 moderately clouded; others nearly clear; all good precipitate.	No. 5 moderately clouded; large precipitate.	Thin; large precipitate.
Fischer's + peptone + glycerin (4119).	Moderately clouded; no gas.	....do........	Moderately clouded; good precipitate.	No. 5 moderately clouded; others thin.	Same as 11 days.
Fischer's+glycerin (4117).	All slightly clouded; a trifle better than plain Fischer's.	Thinly clouded.	Barely clouded; minute precipitate.	All barely clouded.	Do.
Fischer's + cane sugar + KNO_3 (4114).	Nos. 1 and 5 moderately clouded; others slightly.	Same as 1 day.	Same as 1 day...	No. 5 moderately clouded; No. 1 thin; Nos. 3, 4, and 6 barely; No. 2 clear.	Do.
Fischer's + dextrose + KNO_3 (4111).	Slightly but distinctly clouded.	All thinly and equally well clouded.	Same as 3 days..	Barely clouded; small precipitate.	Do.

TABLE XXIII.—*Experiment 2. Fischer's mineral solution with various additions. Inoculations from fluid coconut cultures Nos. 1 to 6 and Bacillus coli, February 15 to 28, 1910, at 22° C.*

Medium.	1 day.	3 days.	10 days.	13 days.
Fischer's solution (4110).	All but the checks are barely clouded.	Same as 1 day.....	All clear and no appreciable precipitate.	
Fischer's + dextrose + KNO_3 (4111).	All thinly clouded; B. coli is a trifle better than others.	All thinly clouded.	Thinly clouded and small precipitate.	Checks titrated +5; cultures +8.
Fischer's + KNO_3 (4112).	All barely clouded...	.....do............	Thinly clouded and very small precipitate.	
Fischer's + dextrose (4113).	All very thinly clouded.	All just barely clouded.	Thinly clouded and small precipitate.	Check titrated + 5; cultures+11.5.
Fischer's + cane sugar + KNO_3 (4114).	Nos. 1 and 5 moderately; others thin.	Nos. 1 and 5 thin; others barely clouded.	Nos. 1 and 5 moderately clouded; others clear; all small precipitate.	
Fischer's + cane sugar (4115).	All very thin........	All just barely clouded.	No. 1 thin; others clear.	
Fischer's+peptone (4116).	All thin; B. coli a trifle thinner than the others.	All moderate; B. coli a trifle thinner than others.	Well clouded with small white precipitate; no film.	Check titrated + 9; cultures+5.
Fischer's+glycerin (4117).	All barely clouded...	All barely clouded; No. 1 a little more than others.	Slightly clouded; minute precipitate.	
Fischer's+peptone +dextrose (4118).	All well clouded except No. 3.	Nos. 3, 4, and 6 almost clear and heavy white precipitate; others still clouded and good precipitate.	Nos. 1, 2, 5, and B. coli moderately clouded; abundant precipitate; others thin; and abundant precipitate.	Check titrated + 9; cultures+24.
Fischer's+peptone + glycerin (4119).	All are moderately clouded; B. coli a little thinner than others.	Well clouded and large white precipitate.	All are moderately clouded; abundant white precipitate.	Check titrated +8; cultures+21

Bacillus coli was used for comparison in the second experiment, but not in the first. There appear to be no great differences between these organisms and *B. coli*. The experiments show in general that in Fischer's mineral solution alone or when KNO_3 is added the organism barely clouds; when peptone is added moderate growth results; adding glycerin either with or without KNO_3 gives slight growth; when either dextrose or cane sugar, either with or without KNO_3 is added poor growth results; when peptone with either dextrose or glycerin is added moderate growth results.

From this table it will be observed that the organism can obtain its nitrogen and carbon easily from peptone alone, but somewhat better when dextrose is present. It can not obtain any nitrogen from KNO_3, and carbon from glycerin only with difficulty (p. 75). The organism can obtain carbon only with difficulty from either cane sugar or dextrose alone; undoubtedly some nitrogenous substance, such as peptone with either cane sugar or dextrose is necessary for good growth.

MEDIA WITH MALACHITE GREEN.

The use of malachite green as a differentiating medium between *Bacillus coli* and *Bacillus typhosus* has been recommended by Loeffler, according to Kiralyfi,[1] who has also tried it but without success. In view of the variable results obtained by Kiralyfi the effect of malachite green as inhibitory to *Bacillus coli* is not taken here as a diagnostic character. As a matter of fact, notwithstanding that Kiralyfi in some experiments found that a 0.02 per cent solution of malachite green prevented good development of *Bacillus coli* colonies, in the following experiments with the same amount *Bacillus coli* grew well. The only points to be ascertained here were whether *Bacillus coli* and the coconut organism grew equally well, producing colonies of the same form and causing a reduction of the color. The experiments were carried out as follows:

(1) Agar slant cultures with malachite green. In 24 hours the growth was wet shining and irregular, the same as in ordinary agar tubes. The growth appeared slightly greenish, but this may have been due to the medium. After three days all the tubes showed good growth and all were entirely or nearly bleached. Culture No. 5 had entirely reduced the malachite green, but in *Bacillus coli* a very distinct green was still at the bottom. After four days none of the cultures showed even a trace of the green color.

(2) Agar plate cultures with malachite green. The malachite green became entirely reduced on all the plates within three days, *Bacillus coli* accomplishing the reduction more slowly than the others. Plates from cultures No. 5 and *Bacillus coli* showed only round or nearly round colonies. All the other plates showed a mixture of the round colonies and deeply lobed or radiate-branched ones. As some of the smallest colonies

[1] Kiralyfi, G. Ueber den Wert der Malachitgrünnährböden zur Differenzierung der Typhus- und Colibacillen. Centralblatt für Bakteriologie, pt. 1, Originale vol. 42, 1906, pp. 276–279, 371–375.

showed a tendency toward branching, this condition probably is due to the medium rather than to varieties of bacteria.

In all of the cultures *Bacillus coli* and the coconut organism behaved alike in that they grew well and reduced the color of the malachite green.

BEEF AGAR CONTAINING CAFFEIN.

The use of caffein in media as a means of differentiating *Bacillus coli* from *Bacillus typhosus* has been discussed, among others, by Roth,[1] by Birt,[2] and by Courmont and Lacomme,[3] who have not, however, presented evidence of the reliability of this means. The one point in agreement among the workers is that 1 per cent, or sometimes less, caffein in the culture media will completely inhibit the growth of *Bacillus coli*. Under certain conditions it is said also to inhibit *Bacillus typhosus*, but that is of little importance here.

Cultures were made in slant agar tubes containing 1 per cent caffein with all the organisms used in this comparative work. After eight days no sign of growth appeared on any of the slants.

Other cultures on the same medium were made in petri dishes. These were kept for several days, but gave no sign of colonies either in the coconut plates or the *Bacillus coli* plates.

THE MEDIA OF CAPALDI AND PROSKAUER.

Two media,[4] designated Capaldi and Proskauer No. 1 and Capaldi and Proskauer No. 2, are used in these experiments.

No. 1 is made as follows:

Asparagin	grams	0.2
Mannit	do	.2
Sodium chlorid	do	.02
Magnesium sulphate	do	.01
Calcium chlorid	do	.02
Potassium monophosphate [5]	do	.20
Water (distilled)	c. c.	100

No. 2 is made as follows:

Witte's peptone	grams	2.0
Mannit	do	.1
Water (distilled)	c. c.	100

In the first of these media, which is free from albumin, *Bacillus coli* is said to grow well and produce acid freely. The second medium

[1] Roth, Emil. Versuche über die Einwirkung von Kaffeïn auf das Bacterium typhi und coli. Zentralblatt für Stoffwechsel- und Verdauungs Krankheiten, vol. 5, 1904, p. 125; Versuche über die Einwirkung des Trimethylxanthins auf das Bacterium typhi und coli. Zentralblatt für Stoffwechsel- und Verdauungs Krankheiten, vol. 6, January to December, 1905, p. 557.

[2] Birt, C. Caffeine Enrichment Method. British Medical Journal, October 28, 1905, pp. 1110–1111.

[3] Courmont, J., and Lacomme, L. La Caféine en Bactériologie. [Discusses certain distinct uses of caffein as an aid in bacterial diagnosis.] Journal de Physiologie et de Pathologie Générale, vol. 6, March 15, 1904, p p. 286–294.

[4] Muir, Robert, and Ritchie, James. Manual of Bacteriology, p. 329.

[5] Potassium biphosphate, monobasic, Merck, was used by the writer in making up this medium.

contains albumin, and is such that *Bacillus coli* is said to grow well but to produce no acid.

After its constituents are mixed and dissolved each medium is steamed for 1½ hours and litmus solution added; the medium is then made neutral, filtered, tubed, and sterilized.

Tubes were made up according to these formulas and inoculated with the coconut organism and *Bacillus coli*.

TABLE XXIV.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli in Capaldi and Proskauer medium No. 1, at 22° C.*

Culture.	Experiment 1, Apr. 7 to 11, 1910.		Experiment 2, Apr. 13 to 30, 1910.		
	1 day.	4 days.	1 day.	3 days.	17 days.
Check	Light reddish purple.	Light reddish purple.	Light reddish purple.	Light reddish purple.	Light reddish purple.
1	Bright pink gas.	do	Bright pink with tinge of purple.	Purple.	
2	do	do	do	Reddish purple above; light pink below.	
3	do	Somewhat purple in upper third; light red below; precipitate bright red.	do	do	
4	Slight change; no gas.	do	do	Light purple nearly throughout.	
5	No change	do	do	Reddish purple above; light pink below.	
6	Bright pink; no gas.	do	Changed only a trifle.	do	
Bacillus coli	Bright pink; gas.	do	Bright pink with tinge of purple.	do	

TABLE XXV.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli in Capaldi and Proskauer medium No. 2, at 22° C.*

Culture.	Experiment 1, Apr. 7 to 11, 1910.		Experiment 2, Apr. 13 to 30, 1910.	
	1 day.	4 days.	1 day.	3 days.
Check	Light purple blue	Almost clear solution with blue-purple precipitate.	Solution light blue; precipitate deep blue.	
1	Gas; purple-red precipitate; very light color in solution.	Practically colorless solution; purple precipitate.	Abundant gas; solution almost colorless; precipitate reddish purple.	Same as on first day.
2	do	Practically colorless solution; white precipitate.	do	Precipitate partially bleached.
3	do	Practically colorless solution; purple precipitate.	do	Do.
4	do	do	do	Do.
5	do	Practically colorless solution; white precipitate.	do	Do.
6	do	Practically colorless solution; purple precipitate.	do	Do.
Bacillus coli	do	do	do	Do.

Check tubes of these media were treated as follows:

No. 1+acid =bright salmon pink.
No. 1+alkali=deep blue.
No. 2+acid =bright salmon pink.
No. 2+alkali=no change.

In the first medium the culture grew well and produced acid, as shown from the change in the color of the light reddish-purple check to the bright pink cultures, *Bacillus coli* acting in the same way as the coconut cultures. In most cases the color subsequently became bleached and in the upper part a distinct blue (after 17 days). These tubes tested with neutral litmus paper indicated an alkali formation, as the change in color of the culture from bright pink to blue also indicates.

The reaction in medium No. 2 was unsatisfactory. In the check tubes as in the cultures the blue color precipitated in the form of fine particles. This precipitate remained blue in the check, but became a distinct purple in the cultures, and in 17 days was bleached almost white. A distinct, though not striking, change from deep blue to purple took place in the color of the precipitate. This change would suggest some acid formation, although the medium is not supposed to permit of acid formation. The reduction of the litmus is the only striking part of the reaction in medium No. 2.

BEEF BOUILLON OF VARIOUS DEGREES OF ACIDITY.

Table XXVI gives the results of four experiments, showing very little constant difference in the growth of cultures in beef-bouillon media of various degrees of acidity or alkalinity. Any sort of bouillon from −12 to +30 on Fuller's scale seemed to furnish the means for luxuriant growth of the organism. The cultures show a tendency to clear sooner at +23, +25, and +30 than at the lower degrees of acidity.

TABLE XXVI.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli in beef bouillon of varying degrees of acidity or alkalinity, at 22° C., as shown in experiments 1, 2, 3, and 4, February 5 to March 14, 1910.*[1]

Titration (grade of acidity or alkalinity).	1 day, experiments 1 and 2.	3 days, experiments 1 and 2.	4 days, experiment 3.	7 days, experiment 1.
−4.....	All well clouded; Nos. 3 and 6 have thin films (experiment 1).	Nos. 2, 3, 4, and 6 have good films; all well clouded (experiment 1).	All heavily clouded; B. coli has no film; Nos. 2 and 6 good.	All heavily clouded; Nos. 1 and 5 and B. coli have no films.
−6.....	All well clouded with thin films (experiment 2).	Nos. 3, 4, and 6 have good films; all heavily clouded (experiment 2).	All heavily clouded; B. coli and No. 2 have partial films; others none.	
−12....	All well clouded with very thin films (experiment 2).	All heavily clouded with films (experiment 2).	All well clouded; abundant white precipitate; Nos. 1 and 5 have no films.	
+2.....	All well clouded; No. 1 has good film; others heavy precipitate (experiment 2).	All well clouded; good precipitate (experiment 2).	All heavily clouded; B. coli has no film; Nos. 2 and 6 good films.	
+7.....	All well clouded; Nos. 3 and 1 have thin films (experiment 1).	Nos. 2, 3, 4, and 6 have good films; others have thin films, except B. coli (experiment 1).	....	All heavily clouded; Nos. 1 and 5 and B. coli have no films.
+23....	All well clouded; thin films (experiment 1).	All good films; well clouded (experiment 1).	All heavily clouded; good films.	All heavily clouded; good films; large white precipitate.
+25....	All well clouded; No. 1 has good film; others barely perceptible films (experiment 2).	All well clouded; good precipitate; good films (experiment 2).	.....do................	
+30....	Same as +23, only more heavily clouded (experiment 1).	All but Nos. 4 and 6 have good films; all well clouded (experiment 1).	....	All heavily clouded; good films; large white precipitate.

[1] Experiment 1, Feb. 11 to Feb. 25; 2, Feb. 5 to Feb. 25; 3, Feb. 21 to Mar. 14; 4, Feb. 25 to Mar. 14.

TABLE XXVI.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli in beef bouillon of varying degrees of acidity or alkalinity, at 22° C., as shown in experiments 1, 2, 3, and 4, February 5 to March 14, 1910*—Continued.

Titration (grade of acidity or alkalinity).	10 days, experiment 2.	11 days, experiment 4.	14 days, experiment 1.	17 days, experiment 4.	21 days, experiment 3.
−4	....	....	All have good films but No. 1 and B. coli.	....	All well clouded; B. coli has no film and no crystals; all others have crystals.
−6	All well clouded; abundant white precipitate; B. coli has no film; No. 1 thin film; all others have good films.	....	....	....	All heavily clouded; B. coli has no film but many crystals.
−12	All well clouded; abundant white precipitate and with films.	....	....	....	All heavily clouded; Nos. 2 and 6 have heavy films.
+2	All heavily clouded; films and abundant white precipitate.	....	....	....	All well clouded; B. coli has no crystals; all have films.
+7	....	All heavily clouded; all but B. coli have films and crystals.	All heavily clouded.	All well clouded but No. 5, which is partly cleared; all have heavy precipitates; all but B. coli have films and crystals.	
+23	....	....	All but Nos. 2 and 4 heavily clouded.	....	All well clouded; No. 2 has pretty well cleared; all the rest have moderate films; all but B. coli and No. 2 have numerous crystals.
+25	All well clouded; abundant white precipitate and good films.	....	....	....	All well clouded.
+30	....	All heavily clouded; heavy films; No. 1 has crystals.	All heavily clouded but No. 2.	All well clouded; good films and abundant precipitate; Nos. 1, 3, and 4 have crystals.	

DUNHAM'S SOLUTION WITH VARIOUS PROPORTIONS OF SODIUM CHLORID.

Table XXVII, gives the results of three experiments, showing that the growth of cultures 1 to 6 and *Bacillus coli* is good only in those solutions containing 3 per cent or less of sodium chlorid. There was growth in the 7 per cent solution, but it was very slight. The amount of indol produced by the organism as shown in the column for 25 days indicates to some extent the amount of growth in each

of the culture solutions. In solutions containing as high as 3 per cent NaCl a good indol reaction was obtained, the color corresponding to rose-violet tint No. 1, Répertoire de Couleurs, in the light tubes and darker in others.

TABLE XXVII.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli in Dunham's solution with varying amounts of sodium chlorid, at 22° C., as shown in experiments 1, 2, and 3, February 8 to March 14, 1910.*[1]

Medium.	1 day, experiments 1 and 3.	3 days, experiment 2.	5 days, experiment 1.	7 days, experiments 1 and 2.	8 days, experiment 3.
Dunham's solution—					
With 1 per cent NaCl (4137).	All moderately clouded except No. 4, which is thin with flocculence.	....	Moderate growth; small precipitate; thin films.	No. 5 titrated + 2; check titrated + 4.	All moderately clouded; moderate precipitate; no films; Nos. 1 and 2 begin to clear.
With 1.5 per cent NaCl (4138).	All moderately clouded except No. 4, which has a flocculent suspension.	....	Moderately clouded; No. 1 has flocculent suspension; all have thin films.	....	All moderately clouded and moderate precipitate; Nos. 2 and 3 have thin films.
With 2 per cent NaCl (4139).	In experiment 1 same as with 1 per cent; in experiment 3 all are thinly clouded.	....	All but B. coli have thin films; all moderately clouded.	....	All well clouded with moderate precipitate; Nos. 2 and 4 have thin films.
With 2.5 per cent NaCl (4140).	A trifle less clouded than with 1 per cent.	....	....do........	No. 5 titrated + 3; check titrated + 4.5.	
With 3 per cent NaCl (4141).	All are thinly clouded.	....	All moderately clouded; thin films.	No. 5 titrated + 4; check titrated + 5.	All well clouded; small precipitate; No. 2 is beginning to clear; no films.
With 4 per cent NaCl (4159).	All are thinly clouded. B. coli is a trifle thinner than the rest.	All thinly clouded; very thin films.	....	All thinly clouded; small precipitate.	All thinly clouded: small precipitate.
With 5 per cent NaCl (4160).	Nos. 1 and 5 are thin; others are practically clear.	....do........	....	....	All thinly clouded; small precipitate: no films.
With 6 per cent NaCl (4161).	All clear except No. 1, which is very thin.	All thinly clouded; barely perceptible films.	....	All thinly clouded; small precipitate.	Nos. 1, 3, and 4 are thinly clouded; small precipitate.
With 7 per cent NaCl (4162).	All are clear.....	All thinly clouded.	....	Nos. 1, 3, 4, and 6 thinly clouded and small amount of precipitate; Nos. 2 and 5 barely clouded; B. coli appears clear.	Nos. 1, 3, and 4 thinly clouded small precipitate; others barely clouded, with very small precipitates.

[1] Experiment 1, Feb. 8 to Feb. 25; 2 and 3, Feb. 17 to Mar. 14.

TABLE XXVII.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli in Dunham's solution with varying amounts of sodium chlorid, at 22° C., as shown in experiments 1, 2, and 3, February 8 to March 14, 1910*—Continued.

Medium.	10 days, experiment 1.	14 days, experiment 2.	17 days, experiment 1.	25 days, experiment 3.
Dunham's solution— With 1 per cent NaCl (4137).	Moderate growth; small precipitate; thin films.	....	Moderately clouded; abundant white precipitate; No. 6 has brownish precipitate.	Same as 17 days; addition of H_2SO_4 gave no result; $H_2SO_4+NaNO_3$ gave in 1, 2, and 5 bright red, which after shaking became pink; other tubes were copper red.
With 1.5 per cent NaCl (4138).	Moderately clouded; No. 1 has flocculent suspension; all have thin films.	....	Same as with 1 per cent except that No. 3 has brownish precipitate.	Same as 17 days; in indol test, Nos. 1, 2, and 5 rose-violet; the others darker.
With 2 per cent NaCl (4139).	All thinly clouded; small precipitate.	....	Same as with 1.5 per cent except that B. coli has brownish precipitate.	B. coli is thin; others moderate; in indol test No. 1 barely pink; Nos. 2 and 5 bright pink; others dark rose as with 1.5 per cent.
With 2.5 per cent NaCl (4140).	....	....	All except No. 1 thinly clouded; No. 1 moderate; all have abundant white precipitate.	
With 3 per cent NaCl (4141).	All thinly clouded; moderate precipitate.	....	Same as with 2.5 per cent except that B. coli and No. 4 have brownish precipitate.	Same as with 2 per cent.
With 4 per cent NaCl (4159).	....	Nos. 1, 3, and 6 thinly clouded; Nos. 2, 4, and B. coli only slightly clouded.	....	Same as before; in indol test B. coli barely pink; others same.
With 5 per cent NaCl (4160).	....	B. coli and No. 2 are barely clouded; others are thin.	....	B. coli is clear; others as before; in indol test all show a trace of pink.
With 6 per cent NaCl (4161).	....	No. 2 and B. coli are practically clear with very small precipitate; others are thin with small brownish precipitate.	....	B. coli is clear; No. 5 is clear; others thinly clouded; in indol test Nos. 1, 2, and 5 show a distinct pink, others a mere trace of pink.
With 7 per cent NaCl (4162).	....	No. 2 and B. coli are clear with small brownish precipitate; others are slightly clouded with small brownish precipitate.	....	B. coli is clear; in indol test all tubes show a mere trace of color reaction.

USCHINSKY'S SOLUTION.

Growth in Uschinsky's solution becomes moderate in 48 hours, but never heavy.

COHN'S SOLUTION.

Cultures 1 to 6 and *Bacillus coli* show only a very slight indication of growth, with the exception of No. 5, which in one experiment became well clouded. In a repetition by Miss Lucia McCulloch, an associate worker, the same results were obtained. The six coconut strains and four *Bacillus coli* strains were inoculated from agar slant cultures three days old, using one 1-mm. loop. The tubes were incubated at 33° C. No growth occurred in any except coconut No. 5, which formed a heavy pellicle and numerous crystals.

POTATO AGAR.

Excellent widespread, wet-shining, white growth with raised irregular margins in all the tubes within 48 hours.

CARROT AGAR.

Growth on tubes of carrot agar is thin, wet shining, white, but very restricted, never extending over the surface of the medium.

LITMUS-LACTOSE AGAR.[1]

Growth on litmus-agar slant tubes is barely perceptible, thin, transparent, and spreads along the streak. Occasionally it develops into small colonies, in which case it reddens the litmus.

On plates both *Bacillus coli* and the coconut organism form small colonies which redden the litmus and are semitransparent and zooglœa-like.

OXALIC-ACID AGAR.

Growth on agar containing 0.2 per cent oxalic acid is similar to that on litmus-lactose agar, being very slight.

MERCURIC CHLORID.

Solutions of beef bouillon containing different percentages of mercuric chlorid were made up for the purpose of ascertaining how strong a solution this organism was able to withstand. In one experiment none of the cultures were able to survive in a solution containing mercuric chlorid as strong as 1 to 3,000. In another experiment the cultures became heavily clouded when the tubes contained mercuric chlorid in the proportion of 1 to 1,000, as well as in weaker proportions up to 1 to 7,000.

Miss Lucia McCulloch made additional tests as follows: A flask containing 250 c. c. of mercuric chlorid water (1 to 1,000) was inoculated with one 1-mm. loop of the cloudy water in the V of an agar slant culture (48 hours old) of coconut No. 5. After 1 minute of vigorous shaking two plates were poured. At the end of 3, 5, and 10

[1] For description of the use of litmus-lactose agar or gelatin, see Wurtz's "Method for the Differentiation of Bacillus Typhi from Bacillus Coli," Technology Quarterly, vol. 6, 1893, pp. 241-251.

minutes, respectively, other plates were poured. For inoculation one 3-mm. loop from the flask was used.

A similar set of plates was made from 250 c. c. mercuric chlorid (1 to 5,000) and from 250 c. c. sterile water. In all three cases inoculations were of the same amount and from the same culture, and the plates were poured at the same intervals of time. The plates were incubated at 33° C.

After 24 hours the plates poured from sterile water had numerous colonies (about 1,500 in each plate). The plates from $HgCl_2$ 1 to 1,000 had a total of two colonies; the plates from $HgCl_2$ 1 to 5,000, a total of three colonies.

After 9 days no more colonies had developed in the plates from the $HgCl_2$ solutions.

Another experiment with similar strengths of $HgCl_2$ was made with plates poured at end of 20, 30, and 40 seconds.

No colonies appeared even after six days at 33° C. The check plates from sterile water gave 450 to 500 colonies in 24 hours.

MONOCALCIUM PHOSPHATE.

Two solutions, A and B, were made up, each containing 1 per cent peptone, 1 per cent dextrose, 0.5 per cent sodium chlorid and neutral red, and into A was put 1 per cent monocalcium phosphate and into B, 0.1 per cent. The amount of calcium phosphate in normal coconut tissues is 0.05 per cent.

After 1 day: A tubes were clear.
B tubes were well clouded, but had produced no change in color.
After 16 days: A tubes were perfectly clear.
B tubes all moderately clouded with moderate amount of precipitate. No change in color.

PEPTONE SOLUTION CONTAINING ROSOLIC ACID.

Two solutions, A and B, were made up, each containing 1 per cent Witte's peptone and sufficient rosolic acid to make a bright red, and into A was put 1 per cent dextrose.

After 1 day: A tubes were yellow; good growth.
B tubes remain red; good growth.
After 4 days: A tubes were orange yellow; well clouded.
B tubes unchanged in color; well clouded.
After 11 days: A tubes, same as before.
B tubes, same as before.

This experiment was repeated, and the colors in the tubes were compared with the color chart of the Répertoire de Couleurs:

A check tubes were pink.
A culture, tested after 17 days, resembled honey yellow, tint No. 3.
B check, a trifle darker than the culture tubes.
B culture, tested after 17 days, resembled cardinal red, tint No. 1.

Thus in solution A an acid was produced in the presence of the dextrose and in consequence the rosolic acid changed from pink to yellow.

In solution B, on the other hand, where no sugar was present, no acid was produced, and hence practically no change in color of the medium.

ALBUMIN.

Tubes were made up containing the white of eggs. In order to prepare them the surfaces of the eggs were sterilized in mercuric chlorid and some of the albumin drawn out through the broken surface by means of a sterile pipette and put into sterile test tubes. To several tubes was added a small amount of sterile dextrose. The tubes were then allowed to stand a week to ascertain if they remained sterile, and then inoculated.

The cultures were examined after 20 days incubation and appeared as follows:

No. 6: With sugar; moderately clouded; no odor.
Bacillus coli: Without sugar; clouded; no odor.
No. 3: With sugar; thinly clouded and small precipitate; no odor.
No. 1: And all others with sugar; a little clouded, but the albumin did not appear in any way to be affected.

Transfers were made into beef bouillon from these tubes to ascertain if the organism was still living. After 30 days all the transfer tubes were well clouded. There was a slight clouding in the check tubes themselves, due to the fact that the albumin from the egg is not perfectly homogeneous, and in consequence it was difficult to tell whether growth actually took place or not. In some cases there appeared to be distinct clouding, but in no case was there any evidence of disintegration of the albumin as evidenced by an odor. At no time was there any odor other than that of a fresh egg. It appears probable that the coconut and *Bacillus coli* organisms do not have the power of disintegrating albumin to any appreciable extent.

SUCCINIC ACID.

In a chemical analysis of a peptone-dextrose medium in which the coconut organism had been grown for some days it was found that an abundance of succinic acid was formed. In order to ascertain if it was the production of this acid that inhibited long growth of this organism, a culture solution was made containing 1 per cent peptone and 1 per cent dextrose plus 0.5 per cent succinic acid in one case and plus 1 per cent acid in another case. It was found that even after incubation for 21 days no growth resulted in either medium. Weaker acidities were then tried, as it was thought the organism could not grow when so large an amount of acid was present.

November, 1910, Miss Lucia McCulloch made the following additional tests: A medium containing 1 per cent peptone, 1 per cent dextrose, and 0.1 per cent succinic acid was inoculated from slant-agar cultures 3 days old of the six coconut strains and four *Bacillus coli* strains. Another medium containing 1 per cent peptone, 1 per cent dextrose, and 0.05 per cent succinic acid was inoculated from the same agar cultures. One 1-millimeter loop was used for inoculation and the tubes were incubated at 33° C. At the end of 48 hours a very moderate growth appeared in all the cultures, represented by thin clouding, flocculent particles, and precipitate; no pellicles. Seemingly there is no particular difference in growth in the two media. After 10 days the medium containing 0.05 per cent succinic acid was moderately cloudy, while that containing 0.1 per cent succinic acid was much clearer. The amount and character of the precipitate in the two media are very similar.

After 18 days the 0.05 per cent succinic acid was still cloudy while the 0.1 per cent succinic acid was practically clear. There seemed to be slightly more precipitate in the weaker acid medium.

COCONUT CYLINDERS.

Small pieces of firm coconut tissue from the petioles of leaves were placed in test tubes and a solution of 1 per cent dextrose was added in amounts to cover the lower half of the cylinder. The sugar was for the purpose of facilitating the growth of the organism, the tissues used being too hard to furnish much nutriment.

After 1 day at 37° C.: Growth in each tube indicated by clouding of the liquid.

After 2 days: The liquid and pieces of coconut much discolored. Slow growth on some pieces, blackening and reddening of others.

After 10 days: Check, liquid clear; cylinder hard.

- *Bacillus coli* a, cylinders blackened—not softened, nor slimy.
- *B. coli*, one cylinder soft, but not disintegrated; microscopic examination of section shows no change.
- No. 4, brown, slimy growth on a portion of the cut surface of the cylinder which is reddish black; portion of the side blackened; by squeezing the cylinder drops of reddish thick liquid are forced out; no soft rot; the cylinder remains firm.
- No. 6, blackening of the tissues as in others; also a rotting of the soft tissues.
- No. 2, abundant orange-yellow precipitate and same brown slime as in No. 4; tissue woody, not soft rotted.
- No. 3, same abundant orange-yellow precipitate as in Nos. 2 and 4; cylinder blackened but not soft rotted.
- No. 5, same as Nos. 2, 3, and 4.
- No. 5 a, same orange-yellow precipitate and same brown slime on cut surface of blackened cylinder.
- No. 1, same abundant orange-yellow precipitate as in others.

This experiment was repeated under the same conditions, but there was only a flocculent clouding which quickly cleared away.

No change appeared in any of the cylinders but No. 1, and that was one of the *Bacillus coli* tubes which blackened the cylinder without showing any growth on the surface. It is evident in any case that such woody tissues of the coconut tree furnish a poor medium for the growth of the organism.

TEST 2 OF D. RIVAS.[1]

One c. c. of a 10 per cent solution of NaOH and 1 c. c. of a 50 per cent solution of H_2SO_4 are added to 5-hour cultures incubated at 37° C. in neutral sugar-free bouillon. A purple color resulting from the addition of the NaOH and H_2SO_4 is the test. The color is said to appear upon the addition of acid and to be discharged upon the addition of an alkali in excess, and is not produced in the presence of sugar. The reaction is thought to be closely allied to indol production and is dependent upon the action of the bacteria upon some proteid substance. Experiments were conducted as follows:

1. Neutral bouillon cultures grown at 37° C. Tubes inoculated at 10 a. m. on March 17 and tested at 4.30 p. m. of the same day. No purple coloration appeared either then or after allowing the tubes to stand 16 hours.

2. Sugar-free neutral bouillon tubes were inoculated at 11 a. m. on April 12 and tested at 4 p. m. The tubes were moderately clouded, but no purple color appeared on the addition of the reagents. This bouillon was made sugar-free by growing *Bacillus coli* in it and then filtering, titrating, retubing, and sterilizing. The tubes after sterilization titrated zero on Fuller's scale.

3. Sugar-free bouillon as before. Tested after three days, but no purple reaction appeared, although the tubes containing the reagents were allowed to stand 48 hours.

The failure of the cultures, both *Bacillus coli* and those of the coconut, to respond to this test is not clear. Possibly bouillon only normally free from muscle sugar should have been used.

PEPTONE WITH LEVULOSE, GALACTOSE, AND MANNIT IN FERMENTATION TUBES.

Table XXVIII shows that all the coconut cultures grow well in levulose, galactose, and mannit, and at the same rate as *Bacillus coli*. The gas production in levulose averaged in 15 days 15 mm.; in galactose 35 mm.; and in mannit 25 mm.

---

[1] Rivas, D. Contribution to the Differentiation of Bacillus Coli Communis from Allied Species in Drinking Water. Journal of Medical Research, vol. 18, 1908, pp. 81-91.

TABLE XXVIII.—*Growth and production of gas (in mm.) in peptone with levulose, galactose, and mannit in fermentation tubes, February 21 to March 8, 1910, at 22° C.*

Culture.	1 per cent peptone+1 per cent levulose.				1 per cent peptone+1 per cent galactose.				1 per cent peptone+1 per cent mannit.			
	2 days.	3 days.	4 days.	15 days.	2 days.	3 days.	4 days.	15 days.	2 days.	3 days.	4 days.	15 days.
Coconut 1	(1)	11	20	51	[2] 16	26	35	69	[3] 0	11	20	32
Coconut 3	[3] 8	18	20	20	[4] 0	21	30	37	(5)	16	22	28
Coconut 4	(6)	14	16	14	[4] 0	22	30	33	(5)	15	20	26
Coconut 5	[3] 10	15	17	14	[3] 15	27	35	32	[3] 17	20	22	22
Coconut 6	(7)	11	15	15	(8)	24	33	37	[4] 8	16	22	23
Bacillus coli	(9)	16	19	15	[3] 11	18	22	43	(9)	16	19	22

1 Well clouded in both ends; a few bubbles of gas.
2 Well clouded in open end; thin in closed end.
3 Well clouded in both ends.
4 Moderately clouded in both ends.
5 Moderately clouded in both ends; three small bubbles of gas.
6 Well clouded in both ends; one large bubble of gas.
7 Well clouded in open end; moderate in closed end; many small bubbles of gas.
8 Well clouded in both ends; a few large bubbles of gas.
9 Well clouded in both ends; many small bubbles of gas.

KASHIDA'S LITMUS-LACTOSE AGAR.

Kashida's medium[1] consists of bouillon containing 1.5 per cent of agar, 2 per cent of milk sugar, 1 per cent urea, and 3 per cent of tincture of litmus. The culture medium should be blue. When liquefied, inoculated with the colon bacillus, poured into petri dishes, and allowed to stand 16 to 18 hours in the incubator, the blue color passes off and the culture medium becomes red. If a glass rod dipped in HCl be held over the dish, vapor of ammonium chlorid is said to be given off. The typhoid bacillus produces no acid in this medium, and there is consequently no change in color.

TABLE XXIX.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli on plates of Kashida's medium, April 14 to 18, 1910, at 22° C.*

Culture.	2 days.	4 days.
Coconut 1	Numerous round, wet-shining colonies; plate blue.	Densely sown with minute colonies; plate blue.
Coconut 2	Numerous roundish, wet-shining colonies; plate somewhat reddened.	Numerous large bluish, wet-shining colonies; agar blue.
Coconut 3	do	Numerous large slightly pinkish colonies; agar reddened all over.
Coconut 4	Like culture 2 only with larger colonies	Almost entirely reddened.
Coconut 5	Like culture 2	Medium entirely reddened.
Coconut 6	Like culture 2 only with larger colonies	Portion of medium reddened; portion still blue.
Bacillus coli	Only two colonies of distinct size; many minute ones; agar partly reddened.	Few colonies have reddened the medium.

A glass rod dipped into hydrochloric acid which was not fuming was held over each of the plates, and in the cases of cultures 1 and 2, which were still blue, white fumes arose from the hanging drop. In none of the other plates, all of which were entirely red or partly

[1] MacFarland, Joseph. Textbook upon the Pathogenic Bacteria, p. 487.

so, did any fuming take place. As a check a small drop of HCl was held over a solution of ammonia which was not fuming, and from the hanging drop fumes arose.

TABLE XXX.—*Growth of coconut cultures Nos. 1 to 6 and Bacillus coli on plates of Kashida's medium, April 21 to 25, 1910, at 37° C.*

Culture.	1 day.	2 days.	4 days.
Coconut 1	Shows a reddening; several colonies on one side.	A number of colonies; some on the blue side; some on the red.	Same as on 2d day.
Coconut 2	Slight reddening on one side; no distinct colonies.	Number of colonies; plate all red and partly bleached.	Do.
Coconut 3	Reddened on one side; about 20 pink colonies surrounded by a pink halo.	Number of colonies; part reddened and bleached, part still blue.	Do.
Coconut 4	Slight reddening on one side; no colonies visible.	Several red colonies on red side; a few light brown ones on blue side.	Do.
Coconut 5	do.	A few small red colonies	Do.
Coconut 6	Reddening on one side; several pink colonies.	Several red colonies on red side; several pink colonies on blue side.	Do.
Bacillus coli	Reddened on one side; a number of pink colonies surrounded by a pink halo; in direct light all the plates of this date are a dense blue black.	Number of red colonies; agar all red; some small colonies with reddish-black centers, then a pink zone, and outermost a yellowish zone.	Do.

The reaction, although apparently sometimes not complete, is characterized by the appearance of the red colonies on the medium which changes from blue black to red under the influence of the bacterial secretions. The color of the colonies themselves may first be blue black or a slate color, eventually becoming red if the reaction takes place. There is probably an incomplete union of the litmus with the other constituents of the medium, hence, the unevenness of the reaction, remaining blue on part of certain colonies and becoming red on the other part.

The statement of Kashida as to the reaction of the drop of hydrochloric acid to the gas arising from the colonies is not clear. If the drop of HCl held over the colonies fumes it is due to the formation of ammonium chlorid. Why this result would not take place with *Bacillus typhosus*, which blues litmus and presumably forms ammonia, is not clear. It seems to the writer that *Bacillus typhosus* would cause HCl to fume as well as *Bacillus coli* and more so. The latter organism, it is true, forms ammonia, but only in small amounts. It reddens litmus, and thus the bulk of the product is an acid. In fact, the foregoing experiments showed no response to this test of Kashida's when the colonies became red. When they remained blue it was probably the result of a failure to produce an acid, and of the positive production of an alkali.

REMY'S SYNTHETIC MEDIUM.[1]

Remy uses an artificial medium approximating a potato in composition, but without dextrin or glucose. The composition is as follows:

Composition of Remy's synthetic medium.

	Grams.
Distilled water	1,000.0
Asparagin	6.0
Oxalic acid	.5
Lactic acid	.15
Citric acid	.15
Disodic phosphate	5.0
Magnesium sulphate	2.5
Potassium sulphate	1.25
Sodium chlorid	2.00

All the salts excepting the magnesium sulphate are powdered in a mortar and introduced into a flask with the distilled water. Thirty grams of Witte's peptone are then added and the mixture heated in the autoclave under pressure for 15 minutes. As soon as removed the contents are poured into another flask into which 120 to 150 grams of gelatin have previously been placed. The flask is shaken to dissolve the gelatin, and the contents are then made slightly alkaline with soda solution. The mixture is again heated in the autoclave at 110° C. for 15 minutes, then acidified with a one-half normal solution of sulphuric acid, so that 10 c. c. have an acidity neutralized by 0.2 c. c. of one-half normal soda solution. This acidity is equal to 0.5 c. c. sulphuric acid per liter. After shaking the flask is placed in a steam sterilizer for 10 minutes, then the solution is filtered, and the acidity of the medium verified and corrected if necessary. Finally the magnesium sulphate is added, dissolved, after which the medium is tubed and sterilized by the intermittent method.

At the moment of using, 1 c. c. of a 35 per cent solution of lactose and 0.1 c. c. of a 2.5 per cent solution of carbolic acid are put into each tube.

Upon this medium the *Bacillus coli* colonies are said to be yellowish brown, the typhoid colonies bluish white and small. Fine bubbles of gas from the fermentation of the lactose often occur about the *Bacillus coli*.

Plates with Remy's medium, April 14 to 18.

Two days: No. 4, densely occupied by tiny white colonies. The other coconut plates and *Bacillus coli* just the same.

Three days: Plates just the same as two days.

Four days: Coconut and *Bacillus coli*. The colonies are very numerous on each plate. Where the medium is fairly thick they appear white, and where it is

[1] Remy, L. Contribution à l'Étude de la Fièvre Typhoide et de Son Bacille. Annales de l'Institut Pasteur, Paris, vol. 14, August, 1900, pp. 555–570.

thin they are colorless or transparent. They do not have a perfectly smooth surface, the tiny colonies, especially, appearing more or less conical. All of these plates are identical with one another.

Doubtless these plates were too thickly sown for a characteristic reaction. At any rate, the yellowish-brown color said to be produced by *Bacillus coli* was entirely lacking, while on the other hand the slightly bluish color considered characteristic of *Bacillus typhosus* on this medium was seen in the colonies where the medium was extremely thin.

ELSNER'S POTATO MEDIUM.

Cultures were made on Elsner's potato medium several times, but in each instance the medium became liquefied owing to the high temperature, so that no satisfactory results were obtained. Finally the poured plates were put in a temperature of about 15° C. Within two days tiny white colonies appeared. They were rather numerous, so that even after several days they did not become large. The smallest colonies appeared colorless or white, the larger ones a very light brown. The distinct brown color in the colonies, said to be characteristic of growth on this medium, failed to appear. The medium was made up according to the method given in Novy's Laboratory Work in Bacteriology, page 490.

COCONUT ABSORBENT-ORGAN CYLINDERS.

The absorbent organ of the coconut consists entirely of a spongy tissue which by the time the coconut is well sprouted, completely or almost fills the entire nut. In it are enzymes which convert the insoluble food material in the coconut meat into soluble material for the use of the growing plant. This organ is in actual contact with the meat, at least in the upper end, and thus is able to conduct the converted material directly into the young shoots. The arrangement of these parts is seen in Plate XI. In order to see if there was food material in the absorbent organ sufficient for the growth of the coconut organism, cylinders were steamed in the usual way and the tubes were inoculated with cultures of the coconut organism and with *Bacillus coli*. The tubes were then incubated at 37° C. The results were as follows:

After 1 day: All the tubes were moderately clouded and all but coconut No. 1 b and *Bacillus coli* (Hitchings) a and b had produced some gas.

After 2 days: Only a tiny bubble or so of gas in some of the tubes; no signs of rotting of the cylinders.

After 6 days: No gas; moderately clouded; no signs of rotting of the cylinders.

After 27 days: Same appearance; the organisms seem to have been able to grow well in the water but not to affect the tissues of the cylinders.

SEEDLING COCONUT SPLIT OPEN TO SHOW PARTS. *a*, ABSORBENT ORGAN; *b*, COCONUT MEAT.

COCONUT ABSORBENT-ORGAN PLATES.

Pieces of the absorbent organ were sterilized by means of alcohol, mercuric chlorid, and distilled water, and then placed in petri dishes. The plates were then inoculated, but even after eight days there appeared to be no growth on the tissues.

COCONUT-MEAT CYLINDERS.

Cylinders were made in the usual way from coconut meat and placed in test tubes with enough water to cover the lower half of each cylinder. The tubes were sterilized on three successive days by steaming and were then inoculated. The growth resulted as follows:

After 6 days: The submerged parts of the cylinders were pink in the culture tubes and white in the check tubes. The top of the cylinder was dark and translucent. The liquid was moderately clouded.

After 27 days: All the checks were pink under water; coconut Nos. 2 and 5, and *Bacillus coli* (Hitchings, XIV, and VI–11–V–09) were greenish white under water, and the water was of the same color; the others, including *Bacillus coli* (B. A. I.) were dark pink under water; no definite film in any case; the growth appears to have been only moderate.

COCONUT LEAFSTALK-TISSUE PLATES.

Large pieces of leafstalk of both old and very young leaves were sterilized in alcohol, mercuric chlorid, and distilled water and then placed in plates and inoculated.

After 12 days: *Bacillus coli* (Hitchings) on a large hard piece of leafstalk, surface mottled, but no rot.

Bacillus coli (VI–11–V–09) on a very fibrous piece of leafstalk, covered with a brownish slimy mass; not soft rotted to any extent.

Bacillus coli (XIV) on rather young leaf tissues, black, soft rotted as in similar portions of a naturally infected tree; exactly the appearance of the leaf-base rot in the mature tree.

Bacillus coli (B. A. I.) on two slender leaflets became completely dried up.

Coconut 5, one of the pieces of young tissue was black, soft rotted as in typical cases.

No other coconut organism was tried.

COCONUT-WATER CULTURES.

The ordinary water from the ripe coconut was sterilized in tubes and inoculated. All of the tubes became moderately clouded in two days at 37° C., but they produced no gas and did not remain clouded long. In 15 days all were practically clear except coconut No. 1, which was well clouded.

COCONUT-OIL MEDIA.

Coconut oil was pressed out of finely cut coconut meat both before and after cooking, and this was purified by mixing with alcohol and then drying out completely. After purification it was a perfectly

clear oily liquid. Cultures were made into tubes of this material, but in none of them was there the slightest sign of clouding.

DETERMINATION OF CHARACTERISTICS OF THE ORGANISM BY PHYSICAL METHODS.

Optimum temperature.—Cultures in beef bouillon (+15) were placed in different temperatures and it was found that good clouding resulted in 24 hours and heavy clouding in 48 hours at any temperature from 25° to 45° C. Surface films formed more quickly at the higher temperatures, and the bouillon showed an inclination toward clearing sooner than at the lower temperatures. Cultures have remained heavily clouded at 30° C. for one month; at 22° C. (room temperature) for two months and more; at 39° C. for one month and more. The point of most luxuriant growth appears to lie between 30° and 35° C.

Maximum temperature.—The maximum temperature is not known Cultures kept at 46° C. for two weeks became heavily clouded with a good surface film and afterwards gradually thinned, as though having passed their best growth.

Minimum temperature.—Cultures in beef bouillon (+15) were kept at various temperatures ranging from 3° C. up to room temperature. After one month cultures at 4° C. and below showed no clouding. Cultures at 8.5° C. failed to cloud until after one month, when one-third of the tubes became thinly clouded. Cultures at 10° C. clouded slowly and within a week were moderately well clouded.

Thermal death point.—Cultures in beef bouillon (+15) were exposed for 10 minutes in water heated to various temperatures. Cultures exposed to an average temperature of 54.9° C. (variation from 54.4° to 55° C.) for 10 minutes failed to grow. Cultures exposed to an average temperature of 51.6° C. (variation from 51.4° to 51.8° C.) failed to cloud in 24 hours, but in 48 hours showed a retarding of growth, though not inhibition. Cultures exposed to an average of 51.° C. (the variation from 50.8° to 51.2° C.) were moderately clouded in 24 hours.

Another set of experiments was made and the culture in bouillon clouded in 24 hours after an exposure of 10 minutes to an average of 54° C. (varying from 53.85° to 54.05° C.). Cultures exposed to 53.35° C. (varying from 53.20° to 53.40° C.) clouded well in 24 hours as did cultures exposed to 52.80° and 52° C.

The experiment was repeated, and cultures exposed for 10 minutes to an average of 54° C. (53.95° to 54° C.) clouded in 18 hours. Cultures exposed to a temperature of 55° C. (54.85° to 55.15° C.) became lightly clouded in 18 hours and well clouded in 24 hours. Three of the six culture tubes exposed to a temperature of 56° C. (56° to 56.10° C.) clouded in 24 hours, the three remaining tubes

failed to cloud. Higher temperatures were not tried. The thermal death point is at least above 56° C.

A repetition of this experiment gave the following results: The six coconut cultures and four strains of *Bacillus coli* exposed 10 minutes to a temperature ranging from 59.2° to 59.6° C. failed to cloud in 48 hours at 37° C.; the same series exposed for 10 minutes to a temperature ranging from 57.4° to 57.8° C. failed to cloud in 48 hours at 37° C.; the same series exposed for 10 minutes to a temperature ranging from 56.4° to 56.6° C. failed to cloud in 48 hours at 37° C. with the exception of *Bacillus coli* (Hitchings). None of the coconut cultures and only this one strain of the four *Bacillus coli* strains survived this experiment. It is reported in some textbook of bacteriology that 59° C. is the thermal death point of *Bacillus coli*. However that is, it is certain that none of the organisms used survived 57° C. in this experiment. It was seen in the preceding experiment that all the coconut cultures exposed to a temperature of 54.85° to 55.15° C. grew well, and that after an exposure to a temperature from 56° to 56.10° C. three of the six tubes grew well. From these experiments it would seem that the thermal death point of the coconut organisms and of *Bacillus coli* is between 56° and 57° C.

Miss McCulloch carried out the following additional tests in November, 1910: Six coconut and the four *Bacillus coli* strains in newly inoculated beef bouillon were subjected for 10 minutes to temperatures of 56°, 57°, and 58° C., then incubated at 33° C. In 24 hours two of the *Bacillus coli* (B. A. I. and Hitchings) in the 56° C. set were clouded; no growth in the 57° C. set; coconut No. 1 was clouded in the 58° C. set. In 48 hours three of the *Bacillus coli* (B. A. I., Hitchings, and VI–11–V–09) were clouded in the 56° C. set; no changes in the others. In 10 days no further change.

Other experiments were made, trying 55°, 56°, and 57° C. In 48 hours at 34° C. all of the 55° C. set, with the exception of coconut Nos. 4 and 5, were clouded. Three strains of *Bacillus coli* (Hitchings, VI–11–V–09, and XIV) and coconut No. 2 were clouded in the 56° C. set. Coconut No. 1 and two strains of *Bacillus coli* (Hitchings and B. A. I.) were clouded in the 57° C. set. In six days coconut Nos. 4 and 5 were still clear in the 55° C. set. Coconut Nos. 1, 2, and 3, and three *Bacillus coli*, in the 56° C. set clouded. No further change in the 57° C. set.

Desiccation.—Clean cover glasses were sterilized and drops of the cultures were placed upon them, after which they were set away in sterile petri dishes to dry out at room temperature. Cultures dried two days clouded well in 24 hours. Those dried six days clouded but little in the same time. Cultures dried 15 days were still able to cloud the bouillon.

Sunlight.—Agar plates were made and half of each of them covered with black paper; they were then set on ice in direct sunlight, the ice serving to counteract the heat effect of the sun's rays. This experiment was carried on in the middle of January, about 1 p. m., with a somewhat hazy sun. The plates were thickly sown. Those exposed for one hour failed to show any effect whatever from the sunlight, and developed in an apparently normal manner.

This experiment was repeated on February 2, at noon, in bright sunlight, and salt was added to the ice to reduce to a minimum the liability of the sun's heat affecting the organism. Exposures to the direct sunlight were made for 30, 45, 75, 90, and 120 minutes. In 24 hours all the plates showed good growth on the unexposed half of the dish. On the plate exposed for 30 minutes only about half as many colonies appeared on the exposed side as on the unexposed. On the plate exposed 45 minutes the reduction was still greater, but the colonies could not be definitely counted on account of their tendency to coalesce. On the plate exposed for 60 minutes about one-eighth as many colonies appeared on the exposed as on the unexposed side. On the plate exposed 75 minutes no colony appeared on the exposed side. The same condition was true for the plates of 90 and 120 minutes exposure. In 36 hours six submerged colonies were visible on the 120-minute plate, and some were visible on all the others, in addition to the spread of the colonies from the unexposed side of the plate.

INOCULATIONS FOR THE COMPARISON OF THE COCONUT ORGANISM AND BACILLUS COLI.

In earlier pages of this paper it has been shown that a certain organism could produce diseased conditions by artificial inoculations into healthy coconut trees, identical with typical bud-rot. On subsequent pages it has been shown that this coconut organism is practically identical in its cultural features with the common *Bacillus coli.* The next step was to produce conditions similiar to bud-rot by means of inoculations with *Bacillus coli* derived from animals. For this purpose several experiments have been carried out in the greenhouse with coconut seedlings. The coconut organism was inoculated into some seedlings for comparison with the *Bacillus coli* inoculations.

Experiment No. 1.

Inoculations with the coconut organism and with *Bacillus coli* (from animals) were made into coconut seedlings on February 17 from cultures of February 16. At the same time a solution of ammonium oxalate was injected into a seedling. No check inoculations other than this were made at this time.

The inoculations were examined from time to time, and finally on March 7, 18 days after the injection, the material was collected and attempts were made to isolate the organisms inoculated. The methods of procedure for identifying the coconut organism and *Bacillus coli* were the same and were based on some of the characteristic reactions of these organisms. Dolt's synthetic medium No. 1 (a litmus-lactose-glycerin agar, p. 79), litmus milk (p. 94), nitrate bouillon (p. 71), fermentation tubes containing peptone and dextrose with neutral red (p. 80), and, in some instances, gelatin, were used. These media have been recommended by various investigators who have carried out extensive work with *Bacillus coli* in connection with their "board of health" investigations and full discussions of them are given on the pages cited. Following are the results of this experiment:

Inoculation No. 1, with ammonium oxalate: The inoculation point was about the same as with *Bacillus coli*, only drier.

Inoculation No. 2, with *Bacillus coli:* Discoloration for only a short distance from the inoculation hole; a water-soaked discoloration but not appearing like a soft rot.

Inoculation No. 3, with coconut No. 5: Discoloration extended a distance of 4 centimeters from the hole and the tissues appeared under the microscope to be full of bacteria.

Inoculation No. 4, with coconut No. 5: Discoloration appeared for only a short distance about the inoculation hole; discolored tissues appeared under the microscope to be full of bacteria.

On the agar plates poured in the usual way from these diseased tissues there appeared round, white colonies, typical of the coconut organism in the case of the coconut plates; but round, thin, white colonies, some with dentate margins, both typical and atypical forms of *Bacillus coli*, in the case of the *Bacillus coli* plates.

Transfers were made of selected colonies from these plates to litmus milk, and after five days all of the tubes had produced red surface rings, but in only one case (from coconut inoculation No. 3) had the medium turned entirely red.

These cultures were also transferred to agar containing neutral red, to Dolt's synthetic medium, and again to litmus milk. In each case negative results were obtained for both the coconut organism and *Bacillus coli*.

Plates were again poured from dilutions of the original bouillon tubes containing the diseased material, and this time Dolt's litmus-lactose-glycerin agar was used. In five days pink colonies typical of both the coconut organism and of *Bacillus coli* were formed on their respective plates. Twelve out of the fourteen plates poured showed these colonies.

Transfers were made from these pink colonies to nitrate bouillon, and two days afterwards test for the reduction of nitrate to nitrite

showed in tubes from two of the *Bacillus coli* plates, in tubes from three of the plates of coconut inoculation No. 3, and in one of the plates from coconut inoculation No. 4.

Before the nitrate tubes were used for the test transfers were made to beef bouillon and subsequently transfers from these were made to litmus milk. In two days each of the litmus-milk tubes, from nitrate tubes that had responded to the reduction test, showed the typical reddening of the litmus and coagulation of the milk that is found in the coconut organism and in *Bacillus coli.*

The tests for these organisms were not carried out further, it being considered that the typical reaction found in the litmus-lactose-glycerin agar, in the nitrate bouillon, and in litmus milk were sufficient for identification.

The only further means of identification was to make transfers from the original bouillon which contained the diseased matter directly to various media without the preliminary plating out of individual colonies. In this way transfers were made to litmus milk, in which case all but one of the tubes reddened and coagulated the milk; to beef agar containing neutral red, in which case all the tubes produced gas and turned the color of the medium to a canary yellow at the base; and to litmus-lactose-glycerin agar, in which good pink colonies were formed, as in *Bacillus coli* and the coconut organism, and the agar was entirely reddened. These tests were considered sufficient to indicate that the same organisms were to be found in the diseased material as were originally injected into the healthy tissues. It appears from this that not only the coconut organism but also *Bacillus coli* (from animals) is capable of producing a destruction of the heart tissues of the coconut plant. Although this was not altogether a surprise after making the extensive comparison of the two organisms that has been described on previous pages and the close similarity of the organisms that has been shown, yet the fact that *Bacillus coli* or any bacterial organism that is commonly associated with animal life is capable of producing a plant disease was so unexpected that further confirmation was thought desirable. The one inoculation of *Bacillus coli* described in this experiment, while on the face of it appearing to have all the points necessary for verification, yet demands several repetitions before it can be accepted as an incontrovertible fact. To this end further inoculations were carried out.

Experiment No. 2.

Along with the other inoculations just described as being made on February 17 a second injection of *Bacillus coli* (derived from animals) was made into a coconut seedling and likewise another

solution of ammonium oxalate. This work was done in the usual way and left until April 5, at which time (after 47 days) the material was collected and platings from the diseased tissue were made both by the writer and by Miss Lucia McCulloch. The appearance of the inoculations was as follows:

Bacillus coli.—The outermost point of the inoculation was merely a trifle browned and water soaked and not at all extensive. The next inner leaf and the one inclosing the central leaf had uppermost an inoculation hole which was browned and water soaked, but only 8 millimeters in extent. On the other side of this same leafstalk was a soft-rotted white area about 5 centimeters long. The innermost leaf, which was still folded, showed the result of the inoculation extending over a distance of 9 centimeters. The diseased part at the lower end was only slightly browned and dry, the middle was soft rotted and water soaked, and the upper part was considerably blackened. The rot was a typical soft rot, although it had not reduced the tissues to a watery fluid.

Ammonium oxalate.—In the outer tissues this inoculation had no characteristic effect. In the inner tissues the leaf was somewhat blackened and dry. No soft rot was in evidence. The action seems to have been a poisonous one rather than one having any effect in dissolving the tissues.

The isolation of the organism from the diseased material as carried out by Miss McCulloch is described in the following paragraphs:

Young coconut leaf, brown to black with rot at base. Bacteria only moderately abundant as seen by the microscope. Some mycelium found.

Plates poured with ordinary beef agar showed in 20 hours numerous round, white colonies up to 2 millimeters in diameter. Transfers were made to agar and to litmus milk.

In 48 hours the agar colonies which had been white were cream color, opaque, and not quite round. Transfers were made from the agar tubes to fermentation tubes containing 1 per cent peptone water plus 1 per cent dextrose plus neutral red and to tubes containing nitrate bouillon.

In three days the fermentation tubes contained gas to the amount of 2.5 to 3 centimeters and the closed arms were canary yellow. Five out of the six tubes showed this reaction. The nitrate-bouillon cultures were then tested for the reduction of nitrates to nitrites and the same five out of the six tubes responded to the test.

Transfers were made from the five fermentation tubes which produced gas to slant tubes of Dolt's synthetic medium. In two days the medium became reddened and the cultures showed a good pink, wet-shining growth.

Transfers were made from these slant agar tubes to litmus milk and to agar containing dextrose and neutral red.

The litmus-milk cultures made directly from the plates, for the most part, reddened and coagulated. The litmus-milk cultures, from the slant-agar Dolt's medium, likewise reddened and coagulated.

The agar tubes containing dextrose and neutral red after inoculation showed good growth and a subsequent bleaching of the color, but no change to canary yellow. As the reaction is an inconstant one, however, it can not be considered evidence against the identification of *Bacillus coli.*

Thus, in Miss McCulloch's isolations of the organisms she obtained bacteria which coagulated milk, reddened litmus, grew well on Dolt's litmus-lactose-glycerin agar, produced gas, and caused a change to

canary yellow of fermentation tubes with peptone and neutral red, and reduced nitrates to nitrites.

The writer of this paper likewise made attempts to isolate *Bacillus coli* from the same diseased seedling used by Miss McCulloch. The process was similar to that in experiment 1 and showed results as follows:

The agar plates which were poured from the diseased material showed in 24 hours numerous round, white, raised, wet-shining colonies typical of *Bacillus coli*. Some of the colonies were irregular in shape, even to radiate branching, and some were bluish and iridescent in transmitted light, but these are variations often met with in what passes for *Bacillus coli*.

Transfers were made from the various colonies to plain beef-bouillon tubes and thence to nitrate-bouillon tubes. After three days in the nitrate bouillon, tests were made for the reduction of nitrates, and it was found that 6 out of the 13 tubes responded to the test. Of these six, two were from round, white colonies; one from round, white, iridescent; one from blue iridescent; one from radiate branched; and one from an irregular blue iridescent colony. Those cultures which failed to show the reduction were largely from round colonies.

Fermentation tubes containing dextrose, peptone, and neutral red were inoculated, and after two days at 37° C. showed the typical canary-yellow color in the closed arm, together with an average of 35 millimeters of gas. Four out of seven of the tubes tried responded to this test. From these four tubes transfers were made to Dolt's litmus-lactose-glycerin agar slant tubes where all grew well, reddened the agar, and produced good pink growths.

Transfers were made from these same tubes in gelatin and placed in the thermostat at 37° C., where an excellent growth took place. After 48 hours the tubes were placed in an ice box and the medium soon became entirely solidified, showing that no liquefaction had taken place.

From these results it will be observed that the same conclusion may be derived as from Miss McCulloch's platings, that is, that *Bacillus coli* was isolated from the diseased material obtained from an inoculation of *Bacillus coli*.

Experiment No. 3.

On April 14, 1910, two inoculations were made into coconut seedlings with cultures of *Bacillus coli*.

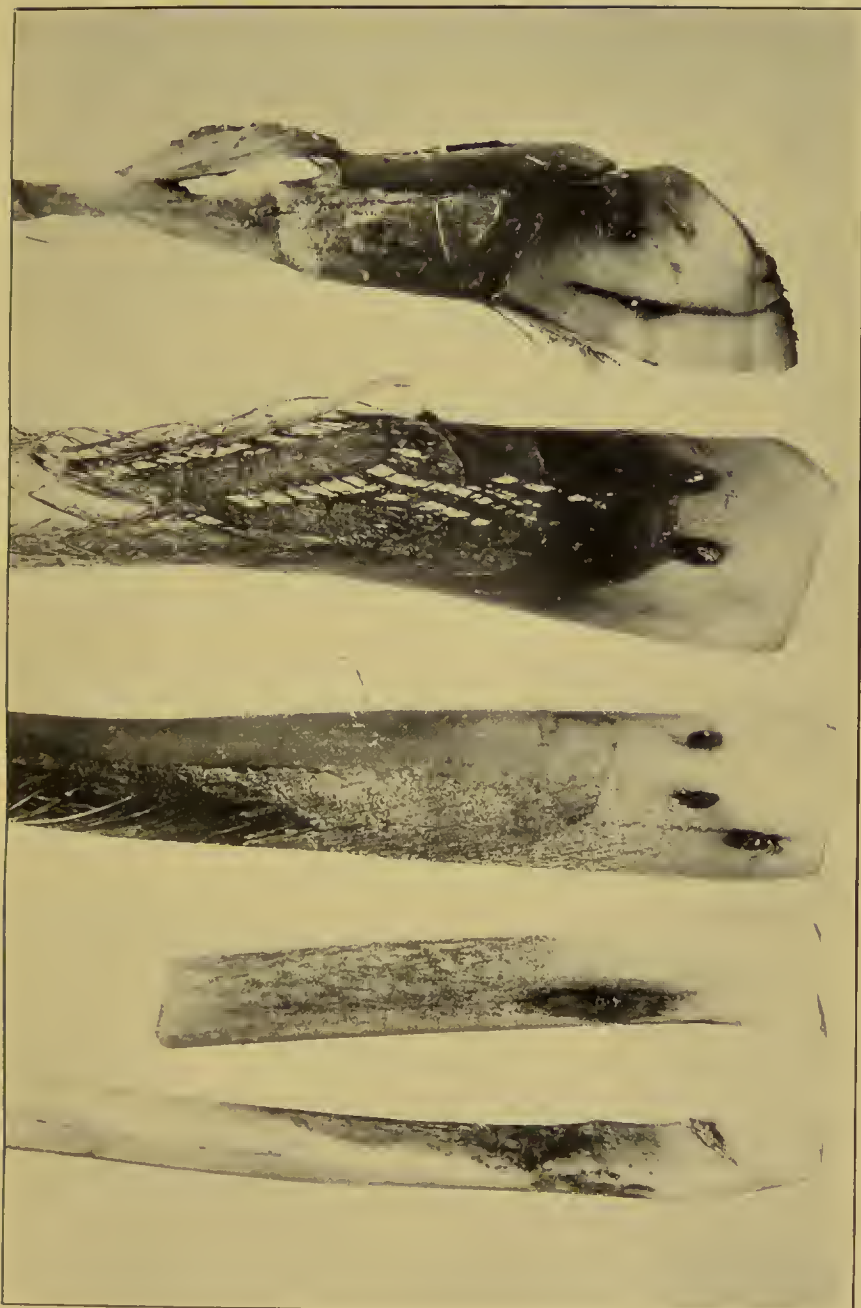
The point of inoculation on the seedling was washed with a solution of mercuric chloride before inoculation.

On May 16, just 32 days afterwards, these two inoculations were cut out and examined. They appeared as follows:

(*a*) Dry, brown discoloration about upper part of hole. At extreme lower end were water-soaked discolorations and slight signs of rot. At the lower part the tissues were considerably split up. The edges of the cracks were yellowed, and in the cracks were masses of what appeared to be bacteria, but little active motility was discernible.

(*b*) Good water-soaked, brown, soft rot extending about 5 centimeters (Pl. XII).

Diseased material from these inoculations was carefully rinsed in alcohol, soaked in mercuric chlorid, and rinsed in distilled water; then, by means of sterile knives and forceps, small pieces were put into test tubes containing beef bouillon and there thoroughly cut up.



RESULT OF INOCULATING BACILLUS COLI INTO COCONUT SEEDLINGS. TIME, 32 DAYS.

These tubes were allowed to stand over night, and on the following day dilutions of the tubes were made in the customary manner and plates were poured, using Dolt's synthetic medium.

In two days every one of the 10 plates showed pink colonies, and where there were more than two or three colonies the agar was entirely reddened. In some plates the colonies were few, while in others they were numerous. For the most part they were round and to all appearances like *Bacillus coli.* The fact that they reddened the litmus and grew well in this lactose medium is good evidence of their identity.

Transfers were made on May 21 from the pink colonies to litmus milk and incubated at 37° C. In 24 hours the four tubes were red, coagulated, and showed abundant whey. Transfers from these litmus-milk tubes were made to nitrate bouillon on May 28 and three days afterwards were tested for the reduction of nitrates to nitrites. Each one of the tubes showed the reduction well.

On June 2 transfers were made from the litmus-milk tubes to fermentation tubes containing peptone and neutral red to test for the canary-yellow color. The tubes were incubated at 37° C. After two days light clouding took place, but no gas formation nor reduction of color. This behavior being entirely contrary to that of *Bacillus coli,* the medium was tested for dextrose, which it should have for the complete reaction. It was found by the use of Fehling's solution that not a particle of reducing sugar was in the fermentation tubes, so a fresh medium containing the peptone, dextrose, and neutral red was made up. These tubes were incubated at 37° C. After 18 hours a small amount of gas appeared, but little change in color. In 24 hours the tubes showed 31 to 34 millimeters of gas, and each one was changed to the canary yellow in the closed end and to a bright red in the open end.

Transfers from each of these four fermentation tubes were made to beef gelatin and incubated for 48 hours at 37° C. At the end of that time all the tubes showed heavy precipitate, several large clots in suspension, and moderate films. The tubes were placed in an ice box and allowed to harden. Eventually each one of these became solidified and showed absolutely no sign of liquefaction of the gelatin.

Thus, in this third experiment the same organism that was inoculated, viz, *Bacillus coli,* was isolated from the decayed tissues.

Experiment No. 4.

On May 7 Miss McCulloch inoculated several coconuts under the supervision of the writer. Strains of *Bacillus coli* (Hitchings and VI–11–V–09) which had not been in the hands of the writer at all were used. The coconuts for this experiment had but recently been

set out after their arrival from a locality in Florida where the bud-rot does not occur. They were just beginning to make good growth. The following notes are from Miss McCulloch:

Seedlings 6 to 12 inches out of nut: At base of stalk a spot was washed with 1:1000 $HgCl_2$, rinsed in sterile water, then with sterile needle a puncture made to heart or center of stalk. The bacterial growth from agar slant was washed off in sterile water and this water (cloudy with bacteria) was injected with a hypodermic needle into the center of the plant. Inoculations Nos. 1 to 4 from agar slants 1 to 4 Hitchings strain of *Bacillus coli;* inoculations Nos. 5, 6, and 7 from agar slants 1, 2, and 3 of VI-11-V-09 strain of *Bacillus coli.* After inoculation the agar from the tubes was taken out on the cotton plug and bound over the point of inoculation. Checks Nos. 8 and 9 were punctured with sterile needle and the binding of agar and cotton put on as with inoculated plants.

Inoculation No. 4, collected June 8: The path of the inoculating needle is brown. In the youngest inner leaf there is a brown, water-soaked area about the inoculation point; extends 1.5 centimeters above and 1 centimeter below the inoculation point. Brownish tissue 1.5 centimeters above used for plating. Tissue was washed in alcohol, mercuric chlorid, and water, and then crushed in the test tubes.

June 10: No colonies on these plates. They have been at 37° C. for 24 hours, and at room temperature for 24 hours. A new set of plates was poured from same tubes.

June 13: A few white colonies only, on original plate; discarded. Same with second set.

Inoculation No. 5: Inoculating needle missed the center of the growth. There is no discoloration about the path of the needle except in the leaf base last punctured, where there is considerable water-soaked, reddish tissue, some of it 3 centimeters from inoculation point. Some of this diseased tissue farthest from inoculation was washed in alcohol, mercuric chlorid, and water, and crushed in test tubes. No organisms responding to *Bacillus coli* tests were isolated.

Inoculation No. 7: The central leafstalk seems unaffected by the inoculation. The base of the leaf just outside this shows discoloration and is slightly water-soaked around the opening made by the needle. All the dark part was cut off from the remainder of the leaf. The hole was laid open—the loose soft part in the opening removed—then the whole discolored part was immersed in 95 per cent alcohol 15 seconds, then in $HgCl_2$ for 2 minutes, and washed in several changes of distilled water for half an hour. The material was then crushed finely in beef bouillon and allowed to stand with frequent shaking for 3.5 hours before plates were poured. After 2 days at 37° C. only a few white colonies appeared on the plate. Plates were again poured on the Dolt's synthetic medium, but as before only white colonies appeared. Plates discarded.

The remaining inoculations of May 7 were examined by the writer, using the customary precautions of rinsing in alcohol and soaking in mercuric chlorid. The results were as follows:

Inoculation No. 1, collected on June 8: The tissues about 2.5 centimeters above inoculation point showed a browning; a rather dry rot. Plates were made in the usual way on June 9 in Dolt's synthetic medium and incubated at 37° C.

June 10: Plate 1 b thickly sown with irregular, luxuriant pink colonies.
Plate 1 c^2, no colonies.
Plato 1 c, numerous pink colonics.
Plate 1 b, Five round pink colonies; one irregular mass.

Plate 1 a, numerous pink colonies.
Plate 1, numerous pink colonies.
Plate 1^2, Four tiny pink colonies.
Plate $1a^2$, Four tiny pink colonies.

Thus, some of these plates suggest the presence of *Bacillus coli.* Four tubes were made from the diseased material, marked thus: No. 1, No. 1 a, No. 1 b, and No. 1 c. From these dilutions were made, marked the same, and from them second dilutions were made, marked No. 1^2, No. $1a^2$, No. $1b^2$, and No. $1c^2$. Plates from these tubes have already been described for one day's growth. In two days they appeared as follows, in the order of their dilution:

No. 1: Well sown with round pink colonies, mostly typical of *Bacillus coli.*
No. 1^2: Five round pink colonies, unlike *Bacillus coli.*
No. 1 a: Many luxuriant pink, irregular, smooth colonies, unlike *Bacillus coli.*
No. $1a^2$: Six colonies, unlike *Bacillus coli.*
No. 1 b: Well sown with round, pink colonies, typical of *Bacillus coli.*
No. $1b^2$: Several colonies; none like *Bacillus coli.*
No. 1 c: Well sown with small, round pink *Bacillus coli* colonies.
No. $1c^2$: Four colonies, unlike *Bacillus coli.*

These notes were made on the plates after transfers, so that some colonies which might have been *Bacillus coli* were destroyed by the needle. Transfers from these plates to litmus milk were incubated at 37° C. All the tubes became reddened and coagulated in 48 hours. Transfers were made from the litmus-milk cultures into nitrate bouillon and incubated at 37° C. for 48 hours. At the end of that time they were tested and all showed reduction of nitrates to nitrites. Transfers were then made from the litmus milk to fermentation tubes containing neutral red and dextrose, and these were incubated at 37° C. After 48 hours every one of the 10 tubes showed the typical greenish-yellow color reaction in the closed arm of the fermentation tube characteristic of *Bacillus coli.* Transfers were made to gelatin and incubated at 37° C. for 48 hours. They were then placed in an ice box to permit hardening. After 10 hours all of these tubes were found to be perfectly firm, thus showing that no liquefaction of the gelatin had taken place. No further tests were made, as it was believed that sufficient had been shown to indicate that *Bacillus coli* was in the tissues into which it had been injected in inoculation No. 1.

Inoculation No. 2, of May 7: The outer sheath was very slightly water soaked about the inoculation hole. The under leaves were rotted only a slight distance; more than in the checks, but scarcely enough to plate out.

Inoculation No. 3, of May 7: The outer sheath was brown rotted 2 millimeters about the inoculation hole on the inner side. Above the hole were numerous brown spots, apparently stomatal infections

from the excess of bacterial liquid inoculated. These tiny brown spots were surrounded by water-soaked areas. The inner part of the tissue was browned and rotted for a distance of 2.5 centimeters. The tissues were not soft rotted. The middle leaves were densely covered with brown water-soaked spots up to 2.5 centimeters from the brown-rotted area. From this inoculation 6 plates were made on June 7 using Dolt's synthetic medium. On June 9 all of the plates showed one or more pink colonies. Transfers were made from the pink colonies to litmus milk and incubated at 37° C. After four days two of the tubes had reddened and coagulated; five had reddened but remained uncoagulated; and one had turned the litmus blue. Transfers to nitrate bouillon showed after 48 hours that all of these cultures except the one which had blued litmus were capable of reducing nitrates to nitrites. Transfers were then made to fermentation tubes containing neutral red and dextrose. These tubes after incubation for 48 hours at 37° C. showed the greenish-yellow reaction in the closed arm as in the case of the same cultures that had both reddened the litmus and coagulated the milk. Those which had only reddened the litmus without coagulating the milk produced a deep-red color in both ends of the fermentation tubes. Thus two, at least, of these cultures appeared to be *Bacillus coli*.

Inoculation No. 6, of May 7, collected on June 8: The outer sheath was brown and water soaked for 8 millimeters about the hole. The inner leaves were brown rotted 25 millimeters from the hole, but there was no soft white rot. No platings were made.

Inoculation No. 8, check: Browning of the tissue was only immediately about the inoculation hole. This discoloration did not extend any appreciable distance. Absolutely no sign of rot or of destruction of tissue.

The results of these inoculations show that all of the cultures produced much more effect on the coconut tissue than did the bare check inoculation; that in some cases there was a distinct rot and that in two inoculations apparently *Bacillus coli* was reisolated. These inoculations were all made with *Bacillus coli*, a strain designated as Hitchings, and made by one unaccustomed to work with the coconut plant—a very important matter. Moreover, the plants were in poor condition for the purpose, as they were just starting a rapid growth which in several cases caused the central leaves to develop into firm, resistant tissue before the rotting effect could take place. The work would probably be more successful if the husk were partly removed about the young shoot and the inoculations made in the thickest part of the stem. As it was, all the inoculations were made outside of the husk in the more or less unsatisfactory

green hardened tissues (Pl. XI) and in plants not making one-quarter as rapid growth as they would have made in the Tropics.

EXPERIMENT NO. 5.

On August 15, 1910, three inoculations were made into coconut trees in Baracoa with a strain of *Bacillus coli* obtained from Dr. Theobald Smith. The three trees were each about 6 years old and were apparently in a perfectly healthy condition, although they were bordering a grove of some 1,200 trees that had just been entirely destroyed by the bud-rot.

On September 28 these inoculations were examined.

Inoculation No. 1 proved to have been made too low. It was below the heart and in the woody tissue. The tissue was entirely rotted about 1 centimeter around the hole from the outside to the interior. On the outer sheaths the brown discoloration extended several centimeters.

Inoculation No. 2 was the same as No. 1. Here also the inoculation was in the wood below the heart.

Inoculation No. 3 was in the soft tissues above the heart. The hole itself was perfectly dry and uninfected in the interior. Extending from the hole upward for 1 meter and only on the inoculated side was the typical soft white rot of the bud-rot disease. The infection was visible on the upper part of the central leaves. There were no insects or other signs of carriers of the disease. It could not be determined if the rot was caused by the inoculation, because, (1) it became more conspicuous from a point 8 centimeters above the hole, but this may have been because of tissues better suited to infection at that point and upward, infectious fluid being injected into all this area; or because (2) by rapid growth the soft injected tissues were carried up beyond the level of that part of the puncture passing through the older tissues. The method of inoculation consisted first in boring a hole to the center of the trunk by means of a 9-millimeter steel bit and then injecting the fluid containing the germs by means of a large syringe. As the terminus of the hole in this case was made into the soft tissues it is very possible that the syringe did not follow the hole throughout, but was pushed to one side in the soft inner tissues. Such a condition could not be determined for the reason that the end of the syringe was small and would make only a very small hole, and the tissues were rotted at this point so that any hole, unless very large, would be indistinguishable. The writer considered the rot due to the *Bacillus coli* introduced by him. On the other hand, it might be claimed that the inoculation failed and that the infection was entirely an outside one. However, if the same kind of organism that was injected could be isolated from the diseased tissues it would

go a long way toward proving the relation of *Bacillus coli* to the disease.

Material from each of these three inoculations was secured, rinsed in mercuric chlorid, then in water, and finally pieces of it transferred by means of sterile knives and forceps to tubes containing Dolt's synthetic medium. These tubes were taken to Washington and there plated out. It was found, by the usual method of isolation described on other pages, that in the case of each of the inoculations, *Bacillus coli*, the same organism that was injected was present in great numbers, although in no case were pure cultures obtained. The results of these inoculations by themselves are rather unsatisfactory, but taken together with the earlier results they afford good evidence as to the relation of *Bacillus coli* to the disease.

Experiment No. 6.

Ten inoculations into coconut seedlings were made with *Bacillus coli* (Theobald Smith XIV) on October 14 in the greenhouse at Washington. Examined on November 10 they showed the following conditions:

Six of the inoculations showed only a slight browning of the tissues about the hole and some water-soaked areas, but no rot nor discoloration of the sheaths.

Two inoculations showed a good brown rot for a short distance about the hole and brown staining for a distance of about 3 centimeters above the hole.

One showed a typical soft wet rot 3 centimeters long and a brown stain 5 centimeters above the hole.

One showed splendid brown soft rot for a distance of 12 centimeters in middle leaves. Outer leaves were well water-soaked and rotted for a distance of 2 centimeters all around inoculation hole, even on the outside sheaths.

No isolations were attempted from any of this series of inoculations.

BACILLUS COLI, THE CAUSE OF BUD-ROT.

Cultures of true *Bacillus coli* have produced infections in the heart tissue of the coconut crown similar to those infections caused by the coconut organisms. Isolations from the *Bacillus coli* inoculations and from the coconut organism inoculations have shown cultures identical in nearly every particular. From the early coconut inoculations, isolations, reinoculations, and reisolations (described on pp. 43–46) the cultures which were obtained have appeared identical in most cases. When difference has existed, it has usually been a matter of degree rather than of kind.

The proof of the cause of the bud-rot will depend for its verity upon the similarity of the various cultures isolated from diseased tissues and upon the constancy of the reactions. Dissimilarity or variation will require satisfactory explanation, or it will count against the statement to be proved. For the proof, so far as inoculations are concerned, the results cited seem sufficient.. There can be no question that good infections were obtained. If now the similarity of the organism injected into the tissues and of the organism isolated from the tissues in various experiments be shown, the cause of bud-rot, and, moreover, *Bacillus coli* as the cause, will be demonstrated. In order to show briefly and in a concise form the similarities and differences among these organisms as ascertained in the cultural work, Table XXXI has been prepared.

TABLE XXXI.—*Summary of characters*[1] *of the coconut organisms and of Bacillus coli.*

Detailed features.	Coconut culture.						Bacillus coli.
	1.	2.	3.	4.	5.	6.	
Morphology of organism	+	+	+	+	+	+	+
Morphology of colony on agar plate, agar stab, streak, gelatin stab, plate	+	+	+	+	+	+	+
Facultative anaerobism	+	+	+	+	+	+	+
Gelatin liquefaction[2]	±	—	—	—	—	—	—
Acid in dextrose	+	+	+	+	+	+	+
Gas in dextrose	±	±	±	±	±	±	±
Acid in lactose	+	+	+	+	+	+	+
Gas in lactose	+	+	±	±	+	±	+
Acid in saccharose	+	+	+	+	+	+	+
Gas in saccharose	+	+	±	±	+	±	+
Reduction of nitrates	+	+	+	+	+	+	+
Pigment production	—	—	—	—	—	—	—
Growth on starch media	±	±	±	±	±	±	+
Acid in glycerin	+	+	+	+	+	+	+
Gas in glycerin	—	+	—	—	+	—	+
Dolt's litmus-lactose-glycerin agar[3]	+	+	+	+	+	+	+
Neutral red:							
With peptone water + dextrose in fermentation tubes	+	+	+	+	+	+	+
With dextrose in agar[3]	+	+	+	+	+	+	+
With lactose in agar	......	......	......	......	+	......	+
With saccharose in agar	......	......	......	......	+	......	+
With glycerin in agar	......	......	......	......	+	......	+
Without sugar in agar	......	......	......	......	+	......	+
MacConkey's bile-salt agar with neutral red:[3]							
In tubes	+	+	+	+	+	+	+
In plates[4]	+	+	+	+	+	+	+
Test 1 of D. Rivas	+	+	+	+	+	+	+
Test 3 of D. Rivas	+	+	+	+	+	+	+
Endo's fuchsin agar[5]	+|	+	+	+	+	+	+
Stoddart's plate medium	+	+	+	+	+	+	+
Hiss's tube medium	+	+	+	+	+	+	+
Sterile milk	+	+	+	+	+	+	+
Litmus milk	+	+	+	+	+	+	+
Production of—							
Indol[6]	+	+	+	+	+	+	+
Phenol[7]	......	......	......	......	—	......	......
H_2S	+	+	+	+	+	+	+
Ammonia	+	+	+	+	+	+	+

[1] Explanation of arbitrary signs used in tho table: + indicates that tho reaction is positive, or merely that the characters are all the same; — indicates that the reaction is negative; ± indicates that the reaction sometimes occurs and sometimes does not; +| indicates that the reaction is a variation in the positivo reaction, which is the samo in all cultures so marked; ±| indicates that there is a variation in the positivo reactions which sometimes occurs and sometimes does not.

[2] For variations in No. 1, see p. 66.

[3] No. 1 tends to bleach.

[4] Nos. 2 and 5 and *Bacillus coli* are slightly different from others.

[5] No. 3 and *Bacillus coli* are slightly different from others.

[6] *Bacillus coli* a trifle moro than others.

[7] This result is questioned.

TABLE XXXI.—*Summary of characters of the coconut organisms and of Bacillus coli*—Continued.

Detailed features.	Coconut culture.						Bacillus coli.
	1.	2.	3.	4.	5.	6.	
Enzymes in milk [1]	—	—	—	—	—	—	—
Growth in nitrogen-free media:							
With ammonium tartrate [2]	+	+	+|	+|	+	+|	+|
With ammonium citrate [3]	+	+	+|	+|	+	+|	+|
With ammonium lactate	+	+	+|	+|	+	+|	+|
With asparagin	+	+	+|	+|	+	+|	+
With sodium asparaginate	+	±|	±|	±|	±|	±|	+
Fischer's mineral solution:							
Alone [4]	—	—	—	—	—	—	....
With dextrose	+	+	+	+	+	+	....
With KNO_3 [5]	—	—	—	—	—	—	....
With cane sugar	+	+	+	+	+	+	....
With peptone	+	+	+	+	+	+	....
With peptone + dextrose	+	+|	+	+	+|	+	....
With peptono + glycerin	+	+	+	+	+|	+	....
With glycerin	+	+	+	+	+	+	....
With cane sugar + KNO_3	+|	+	+	+	+|	+	....
With dextrose + KNO_3	+	+	+	+	+	+	....
Malachite green in sugar	+	+	+	+	+	+	+
Caffein in agar	—	—	—	—	—	—	—
Capaldi and Proskauer:							
No. 1	+	+	+	+	+	+	+
No. 2	+	+	+	+	+	+	+
Dunham's solution:							
With 7 per cent NaCl	+|	+	+|	+|	+	+	—
With 6 per cent NaCl	+|	+	+|	+|	+	+	+
Uschinsky's solution	+	+	+	+	+	+	+
Cohn's solution [6]	—	—	—	—	±	—	—
Potato agar	+	+	+	+	+	+	+
Carrot agar	+	+	+	+	+	+	+
Litmus-lactose agar	+	+	+	+	+	+	+
Oxalic-acid agar (0.2 per cent acid)	+	+	+	+	+	+	+
Coconut cylinders	+	+	+	+	+	+	+
In fermentation tubes:							
Acid from galactose	+	+	+	+	+	+	+
Gas from galactose [7]	+|	+	+	+	+	+	+
Acid from levulose	+	+	+	+	+	+	+
Gas from levulose [8]	+|	+	+	+	+	+	+
Acid from mannit	+	+	+	+	+	+	+
Gas from mannit	+	+	+	+	+	+	+
Kashida's medium	+|	±|	±|	±|	±|	±|	+
Temperatures (degrees Centigrade):							
Optimum	35	35	35	35	35	35	35
Maximum	47	47	47	47	47	47	47
Minimum	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Thermal death-point	57	57	57	57	57	57	57

[1] If present, in only very small quantities.
[2] Nos. 3, 4, and 6 and *Bacillus coli* have much poorer growth than others.
[3] Growth in these tubes varies considerably in amount and color of precipitate.
[4] There appears to be a very slight clouding.
[5] Very slight clouding, if any.
[6] Very slight growth, if any, in all but 5.
[7] No. 1 produced 69 mm. of gas; others ranged from 32 to 43.
[8] No. 1 produced 51 mm. of gas; others averaged 14 to 20 mm.

The importance of the variations shown in the foregoing tables has been discussed under the various experiments. It will suffice to summarize them briefly at this point.

In comparing coconut cultures Nos. 4, 5, and 6 (which are inoculation (6), isolation (5), reinoculation, and reisolation (4) cultures of the same origin) with *Bacillus coli* it is seen that they are all identical until it comes to acid and gas in lactose. In that medium No. 5 and *Bacillus coli* have always behaved alike; Nos. 4 and 6 have always acted similarly to each other and frequently in this medium similarly

to No. 5 and *Bacillus coli.* This variation is the same in saccharose. The growth on starch media shows that all the coconut cultures find difficulty in obtaining sufficient nutriment in such media; but, as before, No. 5 is more nearly like *Bacillus coli.* In glycerin media No. 5 always has reacted like *Bacillus coli* and Nos. 4 and 6 like each other. In all the media, following down to the nitrogen-free media, the reactions of Nos. 4, 5, and 6, and of *Bacillus coli* are identical. In media with ammonium tartrate these cultures are sometimes all alike, but then again Nos. 4, 6, and *Bacillus coli* may differ from 5. It is the same with ammonium citrate and ammonium lactate. In asparagin No. 5 is like *Bacillus coli,* while Nos. 4 and 6 are sometimes similar and sometimes slightly different. In the sodium asparaginate, Nos. 4, 5, and 6 are identical and *Bacillus coli* slightly different. In Fisher's mineral solution with peptone and dextrose, with peptone and glycerin, and with cane sugar and KNO_3, No. 5 is sometimes slightly different from Nos. 4 and 6. In Dunham's solution containing large amounts of NaCl No. 4 shows slightly stronger growth than Nos. 5 and 6 and *Bacillus coli.* In all the other reactions down to Kashida's medium, which gives variable results, the reactions of Nos. 4, 5, and 6 and of *Bacillus coli* are identical.

These experiments have carried these cultures through all the ordinary tests for *Bacillus coli* and many special ones. The few slight variations that have occurred are in no case either sufficient or constant enough to warrant considering any one of the four cultures distinct from the others. As for coconut Nos. 1, 2, and 3, they show almost the same results as Nos. 4, 5, and 6. The origin of these cultures is as follows: No. 6 was isolated from a naturally diseased tree in Cuba on August 7, 1909; transfers from this culture were inoculated into coconut in Cuba on August 12, 1909, and produced a typical soft rot; from this artificially diseased tree coconut No. 5 was isolated on August 24, transfers from No. 5 were then inoculated into a coconut seedling in Washington on September 24, 1909, and a successful infection resulted, from which coconut No. 4 was isolated. Coconut Nos. 1, 2, and 3 represent an isolation in Cuba, an inoculation, reisolation, and reinoculation into a coconut seedling in Washington, and reisolation. Nos. 2, 3, 4, 5, and 6 have been shown to be similar to each other and to *Bacillus coli.* No. 1 has also responded to all the usual tests for *Bacillus coli,* but has in some minor ways shown slight differences. These are probably indications of acquired or lost characteristics and not indicative of a distinct species. The fact that No. 2 was isolated from an infection produced by No. 3 and is identical with it, together with the fact that No. 1 was isolated from an infection produced by No. 2 and is so nearly like it is fair evidence of the identity of these organisms.

From the foregoing remarks on the origin of these cultures it will be seen that Koch's rules for proof of an organism causing a disease have been followed out. The many additional inoculations tend only to corroborate these results.

Thus, having shown, as above stated, that these six cultures are similar to each other and to *Bacillus coli*, the following conclusion seems inevitable: The organism (No. 6) isolated from a naturally diseased tree in Cuba on August 7, 1909, was *Bacillus coli*.

There have been some slight differences as already noted between *Bacillus coli* and the six coconut organisms. They consist in the variable differences found in the nitrogen-free media, with the ammonium compounds, in strength of reaction on starch media, in constancy of reaction in lactose-peptone solution, and in amount of indol produced. In no case is there any definite or constant difference other than in strength of reaction. Taking the reactions as a whole, *Bacillus coli* has at times appeared to differ from the coconut organisms to the slight extent already indicated, differing from them more than they have differed from each other with the exception of No. 1, but not more than the various strains of *Bacillus coli* differ among themselves. This condition indicates that the organisms isolated from the coconut tree are forms of *Bacillus coli*, or at least belong in the colon group, and can not be distinguished from *Bacillus coli* by any of the current methods of bacterial separation.

Moreover, and this appears to the writer to be a decisive point, typical *Bacillus coli* has been shown to be capable of producing typical bud-rot. The conclusion, therefore, seems inevitable that the organism or organisms of the colon group, commonly called *Bacillus coli*, must be considered as the cause of the coconut bud-rot.

In the course of such an extended study on a disease as this has been there naturally occur certain results some of which tend to weaken the case and others to strengthen it. The many successful inoculations and the similarity of the cultures injected and isolated, together with the similarity of many other cultures isolated from diseased coconut material by the writer and earlier by Dr. Smith (p. 142) all tend to strengthen the case. On the other hand, successful inoculations by the writer with cultures of variable appearance rather tend to weaken the argument. These results may be explained by the supposition that other organisms than *Bacillus coli* also produce the bud-rot, or that what passes for *Bacillus coli* includes a group of closely related but not identical organisms. As there is no conclusive evidence for or against such a proposition, this question must remain open. It may be said, however, that the writer does not consider this a probable case. The only alternative in explaining the successful infections with apparently different cultures is the

admission that possibly mixed cultures were used. This alternative is one that no bacteriologist would like to admit, and yet to be perfectly honest it must be considered.

It will be noted that all of the isolations of organisms for inoculation in the case of these successful infections (those with organisms apparently not *Bacillus coli*) were made in Cuba at a relatively high temperature and without the best facilities for work. Bacterial colonies grew luxuriantly in 18 hours, and when the plates had some of the widespreading colonies, they became entirely overgrown in 24 hours, even when sown in the thinnest possible manner. One-half or more of a plate would contain these radiate and rapidly spreading colonies. In the case of the plates which in 24 hours showed apparently only round colonies, after 48 hours some of them showed a tendency to branch. While it does not seem probable to the writer, yet it would seem necessary to admit the possibility in these particular cases of making a transfer from a colony which was apparently a single one, but which in reality consisted of more than one species of organism. The different forms of colonies produced by different organisms and the secretions produced by some species inimical to the growth of others appear to reduce this possibility to a minimum. However, in the case of numerous rapidly growing organisms, all of which are white, wet-shining growths on agar, and varying in their form of colonies, this possibility can not be entirely ignored. The facts of the case, though, do not seem at all to warrant the supposition that the successful infections with organisms apparently different from *Bacillus coli* weaken the claim that *Bacillus coli* itself causes the bud-rot.

It has been shown in foregoing pages that certain organisms were isolated from bud-rot tissues, were inoculated into healthy trees, and carried through a series of isolations and reinoculations, and that certain of the organisms used in these experiments were identical with each other and with *Bacillus coli*. In the very first isolations *Bacillus coli* was unthought of, those colonies being taken which were in the majority and which were thought to be the cause of the disease. Only subsequent work revealed to the writer that the organism was *Bacillus coli*, or at least indistinguishable from it. Later, with inoculations of *Bacillus coli* isolated from animals, a disease similar to bud-rot was produced.

Finally it was decided to search directly in the diseased tissues for *Bacillus coli*. Material was secured on two different occasions. On August 16, 1910, material from two trees was obtained in Baracoa, and transfers of the infected material were made under clean conditions to tubes of Dolt's synthetic medium. Then, in Washington, platings were made and the routine examination for *Bacillus coli*

was made as described on pages 127 to 136. It was found that in the majority of the plates and subsequent cultures made from them true *Bacillus coli* was present as indicated by these tests.

Subsequently, on September 26, 1910, more diseased material was secured near Baracoa, but it was impossible to make cultures at that time. The material was brought to Washington, and after 16 days from the time the material was collected platings were made and *Bacillus coli* isolated. A majority of the colonies on the plates gave the typical reaction, and likewise by the subsequent transfer to litmus milk, nitrate bouillon, neutral red in fermentation tubes, and gelatin the presence of *Bacillus coli* was indicated.

COMPARISON OF BACILLUS COLI WITH VARIOUS ORGANISMS ISOLATED FROM THE COCONUT.

In the early work of the writer many cultures were made from the diseased coconut palms, as has been stated on previous pages. None of these were studied sufficiently in a cultural way to identify them. Dr. Smith also obtained numerous bacterial cultures during his work in Baracoa, but none of them were studied sufficiently to prove them the cause of the bud-rot or to identify them in any way. Studies were made, however, with many of these cultures in such media as litmus milk, nitrate bouillon, sugar peptone, or sugar broth. As these media give characteristic reactions for *Bacillus coli*, the reaction of these various organisms in them will give some indication of whether they are similar to *Bacillus coli* or are decidedly different. A great difference in the cultures would make it difficult to explain so many isolations totally dissimilar. On the other hand some similarity, that is to say similarity so far as tested, would indicate that probably *Bacillus coli* had been isolated in early cultures, but was not identified as such. If some of Dr. Smith's cultures can be shown to be similar to *Bacillus coli*, so far as tested, it will tend to corroborate this paper, which shows that the colon organism is found in the advancing margin of the diseased tissue and is the cause of the diseased condition.

The following are brief notes on some of the early cultures made by the writer:

Culture Cuba B No. 2 of January 2, 1908, produced gas in fermentation tubes with maltose.

Culture Cuba B No. 3 produced gas in fermentation tubes containing dextrose.

Culture Demerara B No. 5 of January 2, 1908, produced gas in fermentation tubes with maltose.

Culture Demerara B No. 6 produced gas in fermentation tubes with dextrose.

Culture Cuba Nos. 1, 2, and 3 of April 9, 1908, reduced the litmus entirely with the exception of a slight reddening at the surface. At room temperature coagulation took place in four days.

Of fermentation tubes of April 16, 1908, with various coconut cultures, 16 out of 24 produced gas in amounts from 5 to 9 centimeters in seven days.

Six bouillon tubes containing cultures from Trinidad on July 23, 1907, showed some gas formation.

Two bouillon tubes of July 12, 1907, from Demerara showed some gas formation.

Five bouillon tubes of July 13, 1907, from Demerara showed some gas formation.

Three tubes of July 29, 1907, from Demerara in litmus milk became bleached and coagulated.

Four tubes on August 7, 1907, from Demerara in litmus milk become reddened and coagulated.

Two litmus-milk tubes of Cuba became bleached.

One litmus-milk tube of Jamaica became partially bleached and another of Jamaica became bleached and coagulated.

Of the cultures of 1909 isolated in 1908, two tubes of 248 Cuba bleached litmus milk and coagulated it. Of all the coconut cultures in use by the writer early in 1909, seven different ones blued litmus milk, seven turned the litmus pink but did not coagulate the milk, and 23 both turned the litmus pink and coagulated the milk. This change from blue to pink in some cases progressed even to bleaching. Some cultures from each of the trees 380, 78, 422, 248, and 153, reddened or both reddened and coagulated the litmus milk as with typical *Bacillus coli*.

Many cultures were isolated in the fall of 1909, among which were the two, 508 N (coconut No. 6) and 505 N (coconut No. 3), carried through the experiments described on previous pages.

Of this lot isolated September 23, 1909, which was designated series N (Table IV, p. 45), many show the *Bacillus coli* reactions.

No. 502 failed to show any reduction of nitrates.
No. 503, two tubes, both showed good reduction of nitrates.
No. 504 failed to show any reduction of nitrates.
No. 505, two tubes, both showed reduction of nitrates.
No. 506, three tubes, all showed reduction of nitrates.

Nos. 502 and 504 failed to show the reaction in nitrate bouillon that is characteristic of *Bacillus coli*. Several cultures, however, were taken from each of the trees and designated by the same tree number, but with some different tube number, and possibly representing different organisms. For this reason other tubes were selected to repeat the nitrate test, with the following results:

Nos. 502, 504, 505, 506, and 508 showed good reduction of nitrates.

Transfers from these cultures were inoculated into trees, produced a disease, and have been studied subsequently, as shown in the foregoing pages. Other cultures were isolated from trees and, in some cases, inoculated, but have not been further studied. The original cultures, however, were grown in nitrate bouillon and show the following results:

Nos. 503, 507, 601, 602, 603, and 604 show good reduction of nitrates.

Thus, it is shown that some tubes of all these original cultures responded to this test as does the colon organism.

Cultures of these organisms were made in litmus milk and showed the following results:

Series N: Nos. 501 and 501a turned litmus milk blue. Nos. 502, 504, 506, and 508 (six tubes) reddened litmus milk.

Series S: This series was isolated from inoculations made with Series N. No. 502 blued litmus milk. Nos. 504, 505, 506, and 508 reddened litmus milk. A large number of tubes of 508 S were all reddened and coagulated.

Of the series R, the isolations from inoculations with series S, cultures were made on litmus-lactose agar with 505 R (coconut No. 1) and 508 R (coconut No. 4). Ten tubes of 505 R all showed reddening of the agar as with *Bacillus coli.* Fifteen tubes of 508 R showed a similar reddening, while the remaining 18 tubes did not. No other of the early coconut cultures were tried on this medium.

In fermentation tubes with dextrose and peptone many of the cultures produced gas.

The production of gas (in millimeters) by cultures of series N at room temperature for nine days was as follows: No. 502, 24; No. 504, 25; No. 505, 21; No. 506, 30; No. 507, none; No. 508, 26; No. 601, 81; No. 602, 29; No. 603, 28; No. 603a, 31; No. 604, 26.

The production of gas by cultures of series S at room temperature for nine days was as follows: No. 503, 21 mm.; No. 504, none; No. 505, 24; No. 506, 64; No. 508, none; No. 508a, 24.

It must be borne in mind that each number represents several tubes derived from as many colonies, and in each one of these tests given only one of the tubes is represented. For example, in the preceding paragraph one tube of 504 failed to produce gas, while 503 and 505, etc., did produce gas. Other tubes of 504 might also have done so if they had been tried.

The foregoing paragraphs show that the writer had early cultures isolated from diseased coconut trees and that some of these cultures resembled *Bacillus coli* so far as tested. It must also be admitted that there were many cultures which gave positive evidence of not being *Bacillus coli.* It will now be of interest to turn to Dr. Smith's cultures obtained by him in 1904.

Of this series the so-called coconut B responds to many of the tests for *Bacillus coli.* In every medium in which it was tested it gave a positive reaction for that organism. The results for coconut B are as follows:

Reduces nitrates.

Produces indol in Dunham's solution.

Produces no nitrous acid in Dunham's solution.

Produces gas in glucose, in cane sugar, in lactose, in maltose, in glycerin, and in mannit.

Coagulates litmus milk.

Besides coconut B, the organisms called coconut D and F responded to the same tests. Moreover, coconut V, AA, BB, and CC, were the same, excepting that there are no records for glycerin and mannit. In addition to these tests, the various organisms were grown in gelatin tubes, and all of the above, with the addition of 15 others, failed to liquefy the gelatin. All of these cultures were grown in litmus milk, and these same strains with some others reddened the litmus and coagulated the milk.

All of the cultures were grown in Dunham's solution and tested for indol after five days. All of the strains indicated above produced indol, B best of all.

The similarity of these organisms in the media used with the coconut organism and *Bacillus coli* is very suggestive of the identity of all.

Of further interest in the comparison of various cultures is the fact that Mr. James B. Rorer has sent to the writer some cultures of organisms isolated by him from diseased coconut palms in Trinidad. These five cultures in litmus milk and in nitrate bouillon are all alike and give the same reaction as the coconut organism, isolated from Cuba, or *Bacillus coli*. They differ, however, in gelatin. This variation seems at once sufficient to consider the organism a different species, but whether the Trinidad form is not a variety of the Cuban organism may well be questioned. It has been noted on a previous page that many of the cultures of 505 R (e. g., coconut No. 1) differed from the others mainly in liquefying gelatin, while *Bacillus coli* does not. It will be noted that the failure to liquefy gelatin is the main difference (at least so far as the arbitrary relationship shown in the chart of the Society of American Bacteriologists goes) between *Bacillus coli* and the soft-rot organism, as shown in the work of Jones, Harding, and Morse.[1] Other differences depend largely upon the ability of the varieties of soft-rot organisms to form acid and gas in media containing carbohydrates. But this is a variation found also in so-called varieties of *Bacillus coli*. The organism isolated by Mr. Rorer appears then to belong rather to the well-known soft-rot types. May it not well be that as there is a variation in production of acid and gas in media containing dextrose, lactose, saccharose, glycerin, and other carbon compounds, both in the case of varieties of the soft rot and in varieties of *Bacillus coli*, there may also be a variation in the production of a proteolytic enzyme as demonstrated in the liquefaction or nonliquefaction of gelatin? It has not yet been shown how this can be other than an arbitrary separation of the two groups. *Bacillus coli*, so far as known, is not able

[1] Jones, L. R., Harding, H. A., and Morse, W. J. The Bacterial Soft Rots of Certain Vegetables, Technical Bulletin 11, N. Y. Agricultural Experiment Station, November, 1909, p. 264.

to produce soft rot in the ordinary vegetables which are affected by various kinds of the so-called soft-rot organisms. It seems to the writer, however, that it has been clearly shown that the colon organism can produce a soft rot in a certain vegetable tissue, namely the coconut bud tissue. Not even the recognized soft-rot organisms can produce this sort of decay in all of the ordinary vegetables; so the mere fact that the colon organism and the so-called soft-rot organism do not affect the same tissues is not sufficient argument for placing them in widely separated groups. The writer does not contend that all these organisms are by any means the same, but that there is at least a very close relationship between all of them. It is probable that the whole group of these organisms represents a class extremely variable, and able in some of its many forms to adapt itself to a great variety of conditions. In this question, of course, hybridization plays no part. It is purely a matter of vegetative changes. Surely there has been enough shown in variation of vegetative parts of flowering plants to warrant the conclusion that the vegetable units resulting from the bacterial division may differ sometimes in their biochemical reactions from the original units. From cultural studies it would seem as though the life processes of the bacteria were even more delicately balanced, and that this balance is more easily overthrown than in the higher plants.

BUD-ROT ATTRIBUTED TO CAUSES OTHER THAN BACILLUS COLI.

With a knowledge of the cause of bud-rot and with a thorough understanding of the effects of the bacterial organism, the source of all the trouble, one may compare more intelligently the various diseases of the coconut palm as reported by different workers. That disease of the tree which is characterized by a rot of the heart of the crown has been attributed to numerous causes. The most important of these which have been seriously proposed by practical coconut growers and by scientific investigators are worthy of some consideration. Of late the general trend of opinion has been to admit the possibility of bacteria producing the rot, but to claim that some other cause was responsible for the presence of the bacteria. Such reasons as soil with too much lime, with too much clay, with too little salt; soil too dry, too wet; insects; and fungi; all of these and other minor reasons for the presence of this disease have been given.

The preceding pages have shown distinctly that the bud-rot may be actually induced by means of a wound inoculation into an apparently healthy coconut tree; in other words that the bacterial organism already described is an active parasite. It is only reasonable to assume that some condition unfavorable to the proper growth of the tree will do much to facilitate the work of the bacterial parasite.

For this reason many of the theories as to the cause of the disease may have a grain of truth in them in that the causes assigned may be auxiliary, though not primary, factors in producing the diseased condition. Such being the case, it is desirable to discuss briefly these factors and the probable relative amount of their influence.

In soils containing too much lime, lack of good drainage is probably the immediate cause of the trouble. Trees growing in such soils are rather slender, and have yellowish leaves, and either fail to bear fruit or produce an imperfect fruit. In addition to poor drainage an excess of lime in an insoluble form may have some direct effect upon the roots which will produce in the crown an appearance similar to drought.

Soils consisting too largely of clay are heavy, cold, and damp. Under such conditions stagnation follows, the roots are not able to absorb water with sufficient facility, and injury results.

The question of the amount of salt (NaCl) desirable in the soil has been much debated. It is claimed by some investigators that a very small proportion is necessary, no more than may be found in an average soil whether near the sea or remote from it. Others maintain that placing about the roots of the tree a small quantity of salt and mixing it with the soil benefits the tree greatly. Whether a reduced quantity of salt would so affect the tree as to render it easy to succumb to the bud-rot is not easy to determine. There has been no work done as yet to ascertain this.

In the case of soils either constantly or temporarily too dry there is certainly a weakening of the vitality of the tree. It is difficult to distinguish this condition from the one in which there is an excess of water. It would seem probable that the latter condition would be the most suitable for bacterial growth. Comparison of the spread of the bud-rot in rainy weather with that in dry weather inclines the writer to say that the rainy weather is more favorable, although the difference is not very striking. The effect may depend not so much upon a large amount of moisture as on an upsetting of the balance of chemical constituents of the tissues by any such untoward conditions as drought or excess of moisture.

In the matter of insects occasioning the trouble in the coconut trees Dr. Carlos de la Torre (p. 22) has maintained that the scale insects covering the stomata of the leaves tend to suffocate the plants, i. e., prevent transpiration, and in that way render the tree susceptible to the disease. It can scarcely be denied that a hindrance to the proper amount of transpiration would seriously affect the health of the trees and possibly in that way furnish an opportunity for the work of the bacteria. From the examination of numerous trees affected with bud-rot, where the scale insects were

either absent or present in such small numbers as to have no serious effect on the transpiration, it is very clear that these insects can not be considered as a primary cause of the bud-rot. Reports[1] have been made on the serious injury and even death of coconut trees in Tahiti and other South Sea islands by these scale insects. It is much to be regretted that the investigator has failed to give a sufficient description of the tissues of the diseased tree to enable a comparison to be made with trees affected by bud-rot.

The claim that insects such as the palm weevil, the rhinoceros beetle, and others are the cause of the bud-rot is frequently made by coconut planters. The effect of such insects is purely local, and even if they are present in great numbers they can have no direct influence in bringing about a rotted condition of the bud. They may, however, possibly play an important part in carrying the bacteria from diseased tissues to healthy ones, the organism gaining entrance through the wound caused by the boring or feeding of the insect.

Not many scientific investigators have definitely ascribed the rot of the heart tissue of the coconut to any particular cause. For the most part they have stated what they have seen in the tissues and suggested what might be the cause. In several cases, however, the diseased condition of the coconut tree has been distinctly said to be due to fungi. The most striking are two late publications, those of Mr. Stockdale and of Dr. Fredholm, one of which has been published widely, in regard to diseases in Trinidad. The work of both of these investigators has already been discussed in detail in another publication,[2] but it seems desirable to repeat it in this connection.

Mr. Stockdale's investigations showed to him two distinct types of coconut disease in Trinidad. In one, which he called the "root disease," the trunk showed a red discoloration toward the outside for a considerable part of its length, and the decayed roots and the petioles were infested with a fungus. Eventually, when the vitality of the tree had been reduced, the terminal bud became involved in a soft rot, and the putrid mass then fell over and the tree died. Mr. Stockdale found, also, what he supposed was bud-rot. In this disease the roots appeared to be healthy, the stem showed no sign of the discoloration, but the bud was involved in a vile sort of bacterial rot, and eventually fell over. In the advancing margin of the rot usually were only bacteria, but in a few cases there was some fungous mycelium. This investigator concluded that the root

[1] Doane, R. W. Notes on Aspidiotus Destructor Sig. and Its Chalcid Parasite in Tahiti. Journal of Economic Entomology, vol. 1, 1908, pp. 341–342. Also Notes on Insects Affecting the Coconut Trees in the Society Islands. Journal of Economic Entomology, vol. 2, 1909, pp. 220–223.

[2] Johnston, J. R. The Serious Coconut Palm Diseases in Trinidad. Bulletin 64, Trinidad Department of Agriculture, 1910, pp. 25–29.

disease is due to fungi and the bud-rot to bacteria, claiming in the case of the root disease that the rotted crown was secondary to the diseased root, but admitted the possibility in the case of the bud-rot that bacteria were the primary cause of the trouble. No experiments were made to prove either the fungous or bacterial nature of either disease. In view of the investigations of the writer, it must be admitted that there have been in Trinidad some diseases answering to Stockdale's description of root disease. As noted on page 33, there were a few cases at Guapo which seemed to correspond to this malady. On the other hand, the trouble in Laventille and Point d'Or (pp. 31–33), so far as the writer could ascertain, was entirely due to bud-rot. That fungous infection might easily take place in the root when the crown is affected is admitted, but it must be denied that the cases of rot in the crown in these two districts were cases in which the rot was secondary and the root disease primary. Actual investigation of the tissues of several trees typically diseased revealed a bacterial rot in the crown and no signs of the root disease. What was true for those trees examined may well be assumed to be true for all the other trees showing exactly similar symptoms.

Mr. Stockdale states [1] that in a tree affected with root disease "it is only a question of time before the terminal bud falls over and becomes a putrid mass, and the palm eventually dies." However, he qualifies this statement in a footnote,[1] as follows:

> When a cocoanut palm is affected by any disease or pest, the terminal bud, in the advanced stages, becomes involved in a rot. This must not be confused with "bud-rot."

These remarks would indicate that their author was not very clear on his subject. In the first place, the statement that "when a cocoanut palm is affected by any disease or pest, the terminal bud, in the advanced stage, becomes involved in a rot" is a most sweeping one to make and is not confirmed by any explanatory notes or experiments. Moreover, the statement is misleading, causing one to think that the ultimate rot is due to the disease or pest, whereas it can only mean that when the tree is so affected or diseased that it dies, then the crown rots, quite as is the case with any dead vegetable tissues when sufficient moisture is present, and this is a truism. Furthermore, this note, taken in connection with the preceding statement in describing the root disease, to the effect that "it is only a question of time before the terminal bud falls over and becomes a putrid mass," is still more misleading.

That diseased coconut trees will rot when they die anyone will admit, but that the terminal bud falls over and becomes a putrid

[1] Stockdale, F. A. Coconut Palm Disease. Trinidad Royal Gazette, Feb. 14, 1907, p. 350.

mass as the result of any disease is untrue. The terminal bud will become a soft putrid mass only in the case of bud-rot. The writer of this discussion has studied closely, by actually ascending the trees and by pushing apart the central leaves, the condition of the bud tissues in many trees and has followed out the changes in individual trees during a period of two years. Some trees were naturally diseased with bud-rot, some by insects, and some were artifically inoculated by making holes 45 centimeters long into the heart tissues and then injecting the organisms. It is possible for the writer to state definitely that miscellaneous diseases or injuries to the tree will not cause a soft, putrid condition of the bud. It is moreover possible to state that, so far as the writer's experiments have gone, only a specific kind of bacteria will produce this soft rot. That Mr. Stockdale found such a condition as he describes in the trees he examined is not questioned. The correctness of his conclusion as to the cause of the condition is, however, much in question. Soft rots may occur in the crowns of trees affected with various maladies, but it is probable from the writer's experiments that the apparent cause of the diseased condition of the tree has been only an accompaniment of the real cause. It is the writer's belief that in those cases of root-rot which had rotting crowns the trouble in the crown was distinct from that in the roots and not to be considered a part of it, i. e., the root disease (whatever its cause) and the bud-rot were two independent diseases in the same tree.

Dr. Fredholm has also made investigations of the coconut-palm diseases of Trinidad (p. 26). He described a serious disease in which the trunk was normal and the roots usually so, while the terminal bud became disintegrated into a sour-smelling, whitish, semifluid mass, which when examined under the miscroscope was seen to be swarming with bacteria. The adjacent tissues out to the petiole bases were traversed by fungous mycelium which Dr. Fredholm believed to be the forerunner of the bacterial rot. He states that he considers Stockdale's root disease and the foregoing disease distinct, chiefly for the reason that he has never found the decay of the roots and the discolored stem present in the affected trees which he examined. To substantiate the claim of the fungous nature of the disease, Dr. Fredholm made fungous inoculations which resulted in small, diseased spots on the leaves (p. 26). These inoculations however, were by no means sufficient to prove the fungous nature of the disease. In order so to affect the tree as to produce a bacterial soft rot in the bud it would actually be necessary to destroy the greater part of the leaves. Dr. Fredholm admits that the soft rot is caused by bacteria, and his claim is that the fungus produces conditions in the tree suitable for bacterial infection. He has ad-

mitted, however, in other paragraphs that evidently the bacterial infection can take place independently of the fungi, for he has found what appeared to be that condition. Since he admits the possibility of bacterial infection without fungi, it is difficult to understand why he considers fungi when they do happen to be present as the forerunners of the bacteria. It would appear as though Dr. Fredholm had called the accompaniment of the disease (fungi in this case) the cause of the disease, assigning the real cause of the trouble, bacteria, to a secondary position. The fact that bacteria both alone and with fungi can cause the trouble, while fungi only in connection with bacteria can produce a similar effect, seems to the writer to demonstrate the primary importance of bacteria. That other organisms may subsequently infect trees diseased with bacteria is of comparatively little importance, while it is of the utmost importance to determine the sole and primary cause of the soft, putrid condition in the crown.

The factors rendering trees specially susceptible to this bacterial rot can not be described now. Only enough has been ascertained to indicate that the bacterial disease is induced by certain conditions; whether they be insect injuries or unhealthy conditions of the tree has not yet been determined.

From the nature of the disease itself, in that it is a soft rot, strong arguments may also be advanced against the probability of its being due to unsuitable soil, climate, insects, or fungi. In no case, so far as the writer knows, has a soft rot of tissues been demonstrated to be due to any condition whatever other than to a few fungi and to bacteria. In the case of fungi it seems to the writer that the only claims to their being the cause of this rot in question have been sufficiently disproved.

Others have maintained that certain bacteria are the cause. Only two of these investigators have indicated at all the organism thought to produce the rot. Dr. Davalos (p. 39) isolated in 1886 what he claimed to be *Bacillus amylobacter*, which he believed to be the cause of the soft rot. He, however, has published no series of experiments to prove this belief. Dr. Plaxton (p. 39) showed to the Institute of Jamaica in 1891, under the microscope, slides of a micrococcus. He believed this micrococcus to be the cause of the rot of the crown, without, however, publishing any experiments to demonstrate the truth of his idea.

It seems to the writer that the symptoms of bud-rot are sufficiently characteristic to distinguish it at once from any other known malady of the coconut palm. If such indications as a falling of the immature nuts, a blackening of the flower spikes, a wilting of the central unfolded leaves, and a soft, putrid condition in the heart are mentioned

by a coconut planter or by an investigator, and the cause of such symptoms is asked, it seems reasonably safe to state that they represent a case of bud-rot and are caused by bacterial action. Such has been the basis in discussing on other pages of this paper many American reports of coconut-palm diseases that have not been personally investigated by the writer. Reports of the disease in the Eastern Hemisphere are discussed on the same basis. If there is a fair reasonableness in doing this with the coconut palm it does not seem at all beyond reason strongly to suspect similar symptoms of disease in other palms to be due to a similar cause. Such is the point of view in discussing the occurrence of the disease on other palms.

OCCURRENCE OF THE DISEASE ON OTHER PALMS.

It is not known positively at present whether or not this bud-rot occurs on other palms than the coconut. Information on the subject is extremely desirable. In Cuba a disease of the royal palm has been noted for some time. Mr. Horne,[1] in 1908, wrote in regard to it as follows:

> The royal palms on the high limestone ridge back of Baracoa were in bad condition and some of them were dying. A similar condition was observed at Banes and at various places, in some of which no bud-rot was known to exist. * * * If royal palms are attacked it is so rarely that probably there is no practical importance to be attached to the matter.

The writer has noted dead and dying royal palms near Baracoa during the past three years. There were not, however, more than 15 or 20, nor did the diseased trees have just the appearance of coconut trees affected with the bud-rot. In the royal palm the central leaves remained healthy longest, while the surrounding leaves gradually turned brown and fell off.

In the summer and fall of 1910 the writer had an opportunity to watch this disease more closely. On August 15 the central leaves and three adjacent ones of a certain tree were left standing, while the remaining leaves were either hanging or had dropped to the ground. Natives stated at that time that the trouble with the tree was due to lightning. On September 29 there was only one upright leaf. On the following morning the entire leaf had blown off, thus furnishing a good opportunity to examine its condition. The column, about 2.5 meters long, composed of the leafstalk sheaths, was intact, but most of the leaves had either bent over or had been broken off just above the sheaths. From this point down the entire column to the base, which represented the growing point, there were great areas of brown, water-soaked, and rotted tissue. These areas were more or less con-

[1] Horne, W. T. The Bud Rot and Some Other Coconut Troubles in Cuba. Bulletin 15, Estación Central Agronómica de Cuba, July, 1908.

tinuous on one side from top to bottom, and their extent at the base was undoubtedly the cause of the column blowing over and falling away from the tree trunk. The rotted areas seemed also to extend from one sheath inward to the next, gradually lessening in extent as the middle was approached. The central tissues themselves were not in the least discolored or rotted. In general, the rotted areas closely resembled bud-rot tissues of the coconut, and the same kind of insects that are found in diseased coconut trees, e. g., earwigs, were abundant. The odor from the rotting tissues, while bad, was not exactly that of bud-rot. The whole aspect of these wilting sheaths was that of fleshy tissue that had been completely cut off from the source of life and was undergoing a normal course of decay. In the coconut palm affected with bud-rot the undiseased tissues are white and the cells are turgid. In the royal palm the unaffected unrotted tissues were white, but the cells were rather flaccid. Because of the resemblance between this disease of the royal palm and the bud-rot some of the tissues were brought to Washington and attempts were made to plate out *Bacillus coli*. All but one of the 23 plates made showed some signs of this organism; the average was about 15 out of 100 colonies, in some plates the proportion of *Bacillus coli* being higher and in others lower. The other organisms were of a very great variety. That *Bacillus coli* was present was demonstrated by transferring the suspected colonies, which had reddened Dolt's medium, (1) to litmus milk, (2) to nitrate bouillon, (3) to fermentation tubes containing dextrose, peptone, and neutral red, and (4) to gelatin. The various cultures responded to the tests as follows:

In litmus milk 22 out of 31 gave typical reactions.
In nitrate bouillon 31 out of 31 gave typical reactions.
In neutral red fermentation tubes 26 out of 31 gave typical reactions.
In gelatin 31 out of 31 gave typical reactions.

Thus, over two-thirds of the tubes gave the customary reactions for *Bacillus coli*. There can be very little question of contamination, for the utmost care was taken to wash and soak large pieces of the diseased material in mercuric chlorid, and then by means of sterile knives portions from the interior of the pieces were removed to the test tubes for plating out. There can be very little question that *Bacillus coli* was in the diseased tissue. It may well be questioned, however, whether this organism was the cause of the disease. Certainly the mere fact of finding a small quantity of these germs in the well-rotted tissues is no proof. Thus, the evidence in regard to this royal-palm trouble being bud-rot is of doubtful value. It should be noted in passing that along the coast near Maravi, 7 miles west of Baracoa, scattered at intervals, about 50 dead or dying royal palms may be seen among many hundreds of healthy ones. It should further be

noted, should this disease ultimately be shown to be the same as bud-rot, that some of the younger royal palms are also affected, and that examination showed on the inside of the leaf sheath honeycombings of the tissues caused apparently by a small brown weevil. Surrounding these injuries to the leaves are brown, water-soaked areas similar to those in the royal-palm tissues already described. Whether these rotted areas may be due either directly or indirectly to the insect injuries has not been demonstrated.

A serious disease of the betel-nut palm and of the palmyra palm has been reported as prevalent in India. The description of the disease of the betel-nut palm as given by Mr. E. J. Butler is as follows:

The first symptoms of the disease appear at the time of flowering. A number of the flowers fall without setting fruits, and their stalks blacken and putrefy. The rot gradually extends along the inflorescences and affects the stalks on which nuts are forming, causing the latter to drop while immature. Very often the damage does not stop here. The flower stalk arises from the axil of the lowest leaf and, therefore, leads directly to the base of the swollen green part of the top of the stem. This green portion consists of a number of leaf sheaths which clasp the young, growing end of the palm, forming thick, protective covering to the growing point. The lowest of these sheaths becomes affected near the point of origin of the flower stalk, and a patch of rot makes its appearance at this point. The sheaths next underlying the first are then attacked, and, since the internal parts are softer than those outside, the rot spreads with increasing rapidity as it approaches the apical bud. When the growing point in the center of the bud is reached it also is destroyed, and the whole head withers and falls off. Not alone, therefore, is the crop lost, but the whole tree is killed, the damage caused in the affected districts being very heavy.[1]

This description answers perfectly to the bud-rot of the coconut. Mr. Butler, however, attributes the disease to a species of Phytophthora. Mr. L. C. Coleman has also studied this disease, but he lays more stress on the infection of the nuts than on that of the bud. He writes:

The real cause is a parasitic fungus which lives in and on the areca nuts.[2] * * * Occasionally it succeeds in making its way into the tissues of the tree top, and in this case the tree is killed, death taking place within a comparatively few weeks.[3]

As the trees in this region extend up to 70 or 80 feet in height, the disease in the top is to be noticed only after it is quite advanced. It is, therefore, difficult to decide just where the preliminary infection takes place.[4]

The tree top pictured shows a decidedly different condition. Here also the nuts have become diseased and have dropped off, but the bunch stalk, especially at the base, appears to be perfectly healthy, nor could any trace of mycelium be found in sections taken from it. On the other hand, the growing point was badly decayed,

[1] Butler, E. J. Some Diseases of Palms. Bulletin of the Department of Agriculture of Jamaica, vol. 5, pts. 2 and 3, 1907, pp. 48–58.

[2] Coleman, Leslie C. Diseases of the Areca Palm. I. Koleroga. Bulletin 2, Mycological Series, Department of Agriculture, Mysore State, 1910, p. 4.

[3] Coleman, Leslie C. Op. cit., p. 13.

[4] Coleman, Leslie C. Op. cit., p. 53.

FIG. 1.—DRAWING FROM MICROTOME SECTION OF DISEASED TISSUES OF BUD-ROT, SHOWING BACTERIA IN STOMATAL CAVITY.

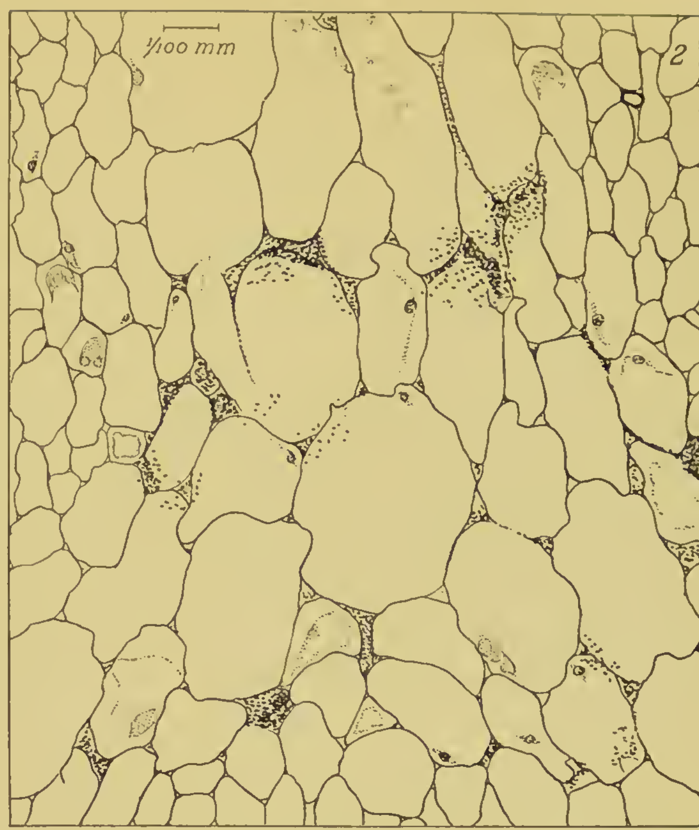


FIG. 2.—DRAWING FROM MICROTOME SECTION OF DISEASED TISSUES OF BUD-ROT, SHOWING BACTERIA BETWEEN THE WALLS OF NORMAL CELLS.

and the course of the decay seemed to have been along the line of the bases of the leaf sheaths which form the protective covering of the tender growing point of the stem.[1]

Coleman made infection experiments both in the nuts and at the growing point of the tree, using Phytophthora for that purpose. The inoculations of the nuts took very easily, as did that of the growing point. In regard to the latter he says:

An examination made two weeks after inoculation showed that the fungus had grown right through the several underlying leaf sheaths and had attacked the growing point.[2]

In view of these inoculations by Mr. Coleman there can be little doubt that he has found the cause of the disease. The resemblance between this betel-nut-palm disease and the coconut-palm disease is, however, most striking, infection occurring as blackening of the tissues either in the flower stalks or in the crown of leaves, or in both; subsequent infection of the growing point; soft rot and death of the tree; in the case of the betel-nut palm or areca palm, Phytophthora believed to cause the disease, although numerous bacteria are present in the diseased growing point; in the case of the coconut palm, *Bacillus coli* proved to be the cause of the disease, although Diplodia is usually abundant on diseased nuts and the upper part of the middle leaves.

In some ways the disease of the royal palm of Cuba resembles more closely the areca-palm disease than it does that of the coconut palm. It is not at all improbable that some fungus may be found to occasion this.

The palmyra palm of India has a disease similar in general aspect to that of the areca palm, but it is said to be due to a species of Pythium. The following is from Mr. Butler:[3]

The earliest sign is the alteration in color of one of the leaves, usually one of those recently expanded toward the center of the bud. This turns white and soon afterwards commences to wither. Other leaves are attacked in turn; the heart of the bud is reached, and the whole top withers and falls off, the last stage being reached only after a considerable time.

The leaf sheaths of all diseased trees are marked by irregular sunken spots in greater or less number. In the earlier stages, and particularly in the inner layers where young ones are often numerous, the spots are white; later on they become brown. They are always sunken and usually have somewhat raised edges. They begin on the outer sheaths and may be traced in through succeeding ones toward the heart of the bud. As the inner layers are softer, the inside patches are often larger than those outside, and may even give rise to new patches which extend out again to the outside sheath. In all cases, however, the first appearance is on the outer sheaths. The earlier patches are dry and either free from any appearance of a parasite on the surface or covered with a white mycelium felt. Very soon a wet rot follows, which extends with great rapidity in the delicate central tissues and converts the whole heart into a foul-smelling mass of putrefaction in which everything is involved, and the original agent

[1] Coleman, Leslie C. Op. cit., p. 54. [2] Coleman, Leslie C. Op. cit., p. 58. [3] Butler, E. J. Loc. cit.

is lost sight of. It is at this stage that the insect grubs referred to make their appearance, possibly attracted by the smell. * * *

It is only in the early stages, before the wet rot starts, that the true cause can be made out. This is a fungus of the genus Pythium, a near ally of the Phytophthora found in koleroga.

In no other palm than the coconut has there been shown to be a rot of the crown due to bacteria. In the royal palm, in the areca or betel-nut palm, and in the palmyra palm there occurs, however, a soft-rotted condition, and in all cases bacteria are present. In the areca and in the palmyra palm the disease is said to be due to fungi. It would be of great value to ascertain if bacteria would not bring about a similar condition. Palm trees are difficult subjects for experimentation, and yet it would seem as though the fleshy part of the crown, i. e., the growing point, furnishes unusually good opportunities for the work of bacteria, especially soft-rot bacteria.

In a recent publication [1] Mr. Butler describes in full the bud-rot of the palmyra and coconut palms as he has found it in India. He made several apparently successful inoculations with *Pythium palmivorum*, one of which was on the coconut palm and the others on the palmyra. It must be noted that the inoculations were not made with pure cultures, at least not pure from a bacteriological point of view. This would seem to lay the results open to question.

MICROSCOPIC STUDIES.

The effect of the bacteria on the tissues can readily be seen by studying infected material under the microscope. Microtome sections stained with carbol fuchsin are best adapted for this purpose.

From the section it is evident that the bacteria may gain entrance through stomata (Pl. XIII, fig. 1) and from the inoculation experiments described on other pages it is known that they also effect an entrance through wounds. The germs multiply at the point of entrance and cause a shrinking of the cells immediately surrounding. They rapidly pass into the interstices and between the walls of normal cells (Pl. XIII, fig. 2), far in advance of the collapsed tissues.

The cells of the fundamental tissues have bordered pits in their walls, or it is possible that they are actually pores. The sclerenchyma cells have greatly thickened walls and in consequence have much deeper pits, which appear in places to be actual canals. These canals pass to the middle lamellæ, but it is not certain that they pass through them. In some of the vessels the pits are so numerous as to give the form of papillæ to the thickened wall. Comparison of the pits of the vessels with those of the fundamental tissues is not to be taken to indicate that they are analogous, but merely to show

[1] Butler, E. J. The Bud-Rot of Palms in India. Memoirs of the Department of Agriculture in India, vol. 3, no. 5, 1910, pp. 221–280.

that there are depressions of some kind in the walls of these two classes of cells. In small, thin-walled cells of the wood parenchyma there appear to be no such depressions.

The bacterial action under these different conditions is according to the structure of the tissue; the thin-walled tissue of the woody parenchyma is completely disintegrated and the vessels which it surrounds are set free (Pl. XIV, figs. 1 and 2). Apparently, in this case the bacteria have a solvent action on the thin walls, which are little or not at all lignified. In the fundamental tissue when young, disintegration also results from the bacterial action. In such cases swarms of bacteria, adhering in groups which are of the shape of the cells, may commonly be seen with no distinct walls intervening. In older parts of the fundamental tissue when the walls have become somewhat lignified no disintegration takes place; nevertheless, the bacteria gain entrance and cause a disintegration of the contents of the cells. Whether this entrance is gained solely through the pits (or pores) or is effected by some solvent action of the bacteria on the walls is not certain. With the disintegration of the contents of these fundamental tissue cells the walls collapse, and a soft, watery mass results. As the infection becomes farther removed from the growing point where the tissues are harder the softening action is lessened. In the trunk below the heart an actual rot, i. e., a softening to the consistency of a thick liquid, takes place for a distance of half a meter or so below the growing point (in one case 1½ meters). This rot does not affect the outer portion of the tree (cortex), but leaves it firmly bound together by its many wood bundles as a shell surrounding and containing the soft mass. Toward the lower part of the rot the bundles in the center of the trunk, as well as those at the periphery, remain unaffected, and at the lowest point there is a discolored area which contains no soft rot whatever. It is frequently possible to obtain a bundle of the fibers a foot or more in length which have been freed from the surrounding parenchyma by the rot. Proceeding from the soft tissues upward similar changes are noticed. As the leaves mature the pinnæ become very membranous and lose their fleshy condition. The epidermis becomes thicker and hardened to such an extent that it is unaffected by the bacterial rot. The middle tissues may be disintegrated, but the epidermis remains a transparent, papery membrane, covering the vascular bundles, or veins. Spots often appear high up in both the mature and young leaves, first as small yellow or brown dots which may gradually spread into long, brown, water-soaked streaks, or may be restricted to small dry areas. In the water-soaked streak, which eventually passes down the leaves to the heart, are swarms of bacteria which cause the slimy condition. Fungi also are frequently present. In the small

restricted dried areas are found both bacteria and fungous filaments. It has not been possible always to prove that fungous filaments were present, but it has always been demonstrated that bacteria were present. This is not conclusive evidence one way or the other as to the cause of the spots. In case the spot remains restricted it is evident that the bacteria have gained no foothold to thrive; but when the spots have elongated and become slimy, then the bacteria are flourishing. Diseased areas may occur not only in the upper parts of the mature leaves but also near the base of the leafstalks and on the adjacent part of the trunk. These spots commonly develop as brown areas with a water-soaked appearance. They vary in size from minute ones to those a decimeter or more in length on the main part of the stalk, or they may extend indefinitely into the strainer when it is in a moist condition. These spots, as a rule, are slightly below the general level of the tissue. If a piece of the diseased leaf tissues, the surface of which has been washed clean, is pressed, a cloudy juice oozes out, consisting of the ordinary juice from the tissues made cloudy by the crushed cell tissues and myriads of bacteria. Hand sections of this tissue are difficult to make, but not impossible. These sections show a general brown staining of the tissues and slight, if any, collapse of the cell walls. The contents are granular in appearance. What appear to be bacteria occur in these cells scattered unevenly throughout the diseased parts; they swarm in some of the cells, but are apparently absent from others. No fungous filaments have been found in these water-soaked areas, although fungous infections frequently take place near the base of the leafstalks. Such places present dry, gray, hardened surfaces with tiny pustules here and there. It often happens that spots occur on the upper part of the middle leaves when the heart is perfectly sound and no bud-rot is apparent. These spots have been described above in that they were said to be brown, becoming dry, and to contain either bacteria or fungi, or both. The diseased spot seldom if ever spreads more than 5 to 8 centimeters unless the leaves are very young or have been injured. If the leaves are young or fleshy the rot will spread downward from the bacterial action, causing typical bud-rot at the heart. Until the leaf where the infection first occurred becomes old and membranous the fungous infection will spread either upward or downward, but it will not spread in a healthy uninjured leaf. The fungi that occur on these tissues are various, but the most common are *Pestalozzia palmarum* Cke. and what has been called *Botryodiplodium* sp. They appear at maturity as an irregular sooty mass on the surface of the leaves, or they break out from slender elongated pustules, or they may appear as tiny black dots in the centers of the dry spots. The Pestalozzia is common on diseased palms and

FIG. 1.—MICROTOME CROSS SECTION THROUGH SMALL LEAF BUD OF COCONUT PALM, SHOWING DISORGANIZATION OF THE TISSUES DUE TO THE ACTION OF THE BACTERIA. BACTERIAL CLUMPS AT BOTTOM AND TOP.

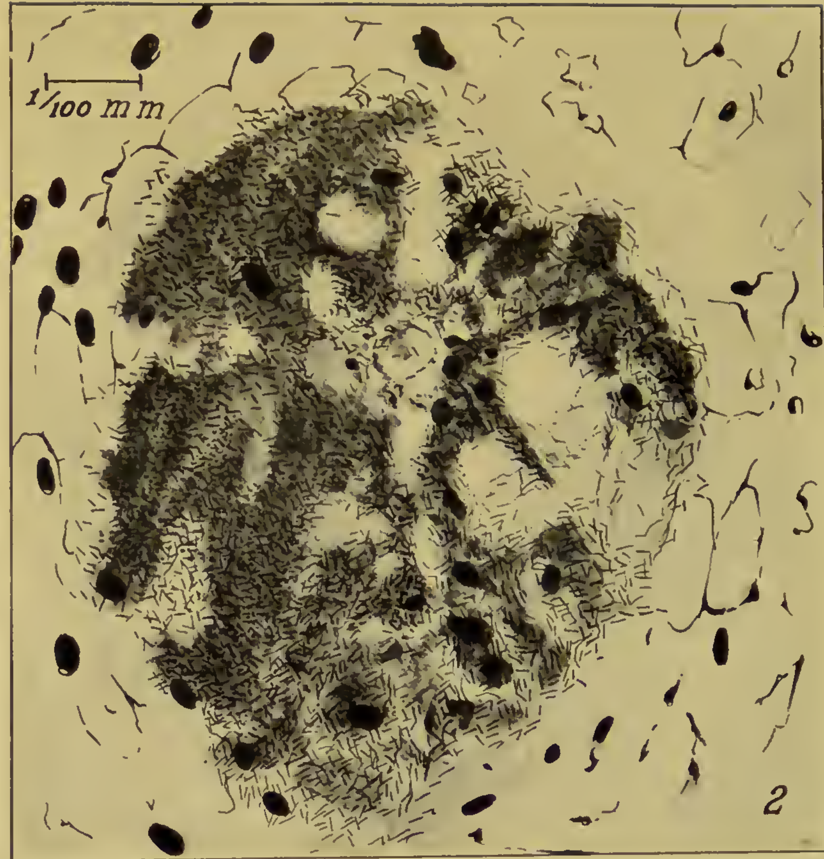


FIG. 2.—ENLARGEMENT OF A PORTION FROM MIDRIB OF LEAFLET.

is easily distinguished. The Botryodiplodium is less distinctive in that its spore form seems to be varied, i.e., there is often either an ovate, olive-black, one-celled spore or an olive, more or less cylindrical, two-celled spore with rounded ends. Either one or the other spore may be colorless or may range from olive to olive-black in color. It is possible that two different fungi are here confused. However that is, it is certain that the forms mentioned are almost invariably found on the diseased central leaves of the coconut.

In addition to the above considerations, tiny brown spots have been rarely found on the surface of the soft tissues near the heart. They appear as slight cracks from which a red, transparent gum oozes. Microscopic sections of these spots revealed no parasites whatever, either fungous or bacterial. The cell corners of the brown area stain deeply, but otherwise no change is apparent. The cause of this has not been discovered.

VALUE OF COCONUT PRODUCTS.

In order to suggest the importance of the coconut industry, statistics as to the exports, imports, acreage, etc., of various countries are here included. It is impossible to obtain full data from all districts, but it is believed that the figures here given are sufficient to indicate partially the great extent of this industry and the very serious nature of any disease so widely distributed and destructive as the bud-rot.

Table XXXII gives the value of the imports of coconuts and coconut products into the United States for the years 1903 and 1904. It will be noted that the items listed are coconuts, oil, copra, prepared copra, and coir fiber. Of these the nuts are obtained almost entirely from tropical America, while some of the other products come largely from the East Indies.

TABLE XXXII.—*Value of coconuts and coconut products imported into the United States.*

Item.	1903.	1904.
Coconuts	$908,242.00	$971,852.00
Coconut oil	2,494,442.20	2,186,161.77
Copra	354,122.00	273,143.00
Prepared copra	134,240.00	135,386.00
Coir fiber	7,098.00	3,462.00
	3,898,144.20	3,570,004.77

Table XXXIII gives the source of a part of the coconuts imported into the United States, as per official reports of exporting countries. The source of the products, oil and copra, are given in volumes entitled Trade and Navigation of the United States, published by

the Department of Commerce and Labor, but the sources of coir are not so readily obtainable; these products come chiefly from the East Indies and the Philippines. Colombia is known to be a large exporter to the United States from the fact that the value of coconuts imported thence into the United States, as shown by the United States Department of Commerce and Labor, is in some years larger than the value of like imports from any other single country. Brazil is said to be a very large producer of nuts, but the exports to all countries in late years are of small importance; there is no record of importations thence into the United States. The places mentioned in Table XXXIII are the ones commonly heard of in the markets.

TABLE XXXIII.—*Source and number of coconuts imported into the United States, by years, 1903–1906.*

[Figures under Bahamas, Honduras, and Porto Rico not verified, as there are no statistics of same on record in the Bureau of Plant Industry.]

Country of origin.	1903.	1904.	1905.	1906.
Cuba [1]	14,579,000	16,733,000	15,501,000	15,968,000
Jamaica [2]	17,670,212	9,364,543	2,258,065	4,651,046
Bahamas	....	....	....	297,850
Honduras	7,868,456	....	....	....
Trinidad [2]	3,951,801	4,245,530	8,508,226	9,054,355
British Guiana [3]	17,258	46,829	561,334	....
Porto Rico	....	....	4,888,053	....

[1] From Estadistica General Comercio Exterior, Republica de Cuba.
[2] Statistical Tables, Colonial and Other Possessions, Great Britain.
[3] From Handbook of British Guiana, 1908.

Coconut trees are found in greater or less numbers in all countries of tropical America bordering the ocean, but they are found by no means along all the coast line. In these countries there are many miles of coast where no such trees are visible. The few countries mentioned in Table XXXIII, together with Nicaragua, Colombia, and Venezuela, of tropical America are the chief sources of the coconuts which are sent to the United States. It is said that the coast of Brazil between the Rio San Francisco and the bar of Mamanguape, a distance of 450 kilometers, is one almost unbroken stretch of coconut trees. In addition, there are some coconuts on the western coast of tropical America, though excepting in Colombia not in great numbers. According to report, South America has 1,000,000 acres in coconut cultivation. Table XXXIV is here included to show the immense importance of the exportation from the East. The data are incomplete and not in all cases comparable, but they suffice to show that the industry is widespread and of very great importance.

TABLE XXXIV.—*Exports, products, or acreage of coconuts of various countries.*

Countries.	Amount.	Year.	Remarks.
Philippines....dollars..	3,819,793	1903	Value of copra exported.
Fiji Islands....	....	1903	Land is covered with coconut trees which, if counted, would number millions.
Samoa Islands....tons..	5,400	1907	Amount of copra exported valued at £77,981.
Marshall Islands....	....	1907	Imported crop.
Tutuila....tons..	500	1907	Copra annually exported.
Solomon Islands....	....	....	Only product yet cultivated.
Federated Malay States....acres..	77,500	1904	Valued at $12,000,000 to $15,000,000.
Portuguese West Africa....	....	1904	Production of copra increasing. Coir fiber has been made for years.
Portuguese East Africa....	....	1901	
German East Africa....	....	1903	German East African Association in Muoa has 200,000 trees; Kjeine, in Togo, 136,000 trees at end of 1901.
New South Wales....hundredweight..	145,510	1907	Valued at £180,787.
Ceylon....acres..	606,134	1886	These exports represent a total number of 565,527,757 nuts.
Oil....hundredweight..	687,623	1903	
Copra....do....	732,015	....	
Desiccated....pounds..	18,384,800	....	
Nuts....nuts..	13,615,589	....	
British India and its dependencies.acres..	480,000	1886	Valued at £8,680.
Mauritius....hundredweight..	7,430	1907	
South America....acres..	1,000,000	1886	

It is reasonable to assume that the use of such products is likely to increase in this country. The present situation in regard to the extent of the coconut industry makes it apparent that the progress of any such widespread disease as the bud-rot should be studied with great care.

Owing to the great distance of the East from the European countries the produce is carried in the form of copra, oil, or coir, rather than as whole nuts, the most common form in tropical America.

No data are available to prove the statement, but it may be gathered from various notes on the subject that European countries are away ahead of America in the consumption of coconut products, particularly that of coconut oil for cosmetics, cooking compounds, butter, medicinal compounds, etc., and coir for mats and ropes.

SUMMARY.

(1) A disease of coconuts has been known for more than 30 years in Cuba. A similar trouble has also caused widespread loss in Jamaica, British Honduras, Trinidad, and British Guiana.

(2) This disease is called the bud-rot, owing to a rot occurring in the bud of the tree. The early symptoms are the yellowing and falling of the leaves and the dropping of immature nuts. Eventually the middle folded leaves bend over and the entire heart of the crown is involved in a vile-smelling soft rot.

(3) The spread of the disease, with the consequent heavy loss, may be very rapid. A single tree may be killed in two months to a year or more after infection, and entire groves may be destroyed in two or three years.

(4) This disease (or a disease with similar symptoms) occurs in many parts of Cuba, both in the eastern and western ends; in western

Jamaica, and in a few cases in the extreme eastern end; in the Cayman Islands; in British Honduras; in Trinidad on the north and west sides; in British Guiana at the mouth of the Essequibo River and at Mahaicony. According to reports it occurs also in the Philippines and in Ceylon; probably in British India, in German East Africa, and in Portuguese East Africa.

(5) This disease was investigated in 1901, at the request of the planters of Baracoa, Cuba, by Mr. August Busck, entomologist of the United States Department of Agriculture. In 1904, Dr. Erwin F. Smith, plant pathologist of the same Department, made further investigations in the same district, and declared the disease to be a bacterial soft rot of the terminal bud. Mr. W. T. Horne, until recently plant pathologist of the Estación Central Agronómica of Santiago de las Vegas, studied the disease in 1906 to 1909, in both the eastern and western districts of Cuba. Mr. W. Fawcett, formerly director of the botanical department of Jamaica, reported the trouble in Jamaica in 1891, and since then has made frequent observations on it. Mr. W. Cradwick, traveling instructor of the same department, has also made studies on the same malady. Prof. F. S. Earle, while on the staff of the New York Botanical Gardens, reported on the disease in Jamaica in 1902. Mr. W. A. Murrill, of the same staff, reported on it in 1908. In Trinidad, Mr. W. Greig called attention to the disease in 1905. Mr. J. H. Hart, formerly director of the botanical station of Trinidad, investigated the trouble in the same year. Mr. F. A. Stockdale, until recently mycologist of the Imperial Department of Agriculture for the West Indies, studied the troubles of the coconut in 1906. Dr. A. Fredholm made a report on the fungous diseases of the coconut in Trinidad in 1909.

(6) The extent and nature of the disease of the coconut were investigated by the writer for the United States Department of Agriculture, in 1907, in Cuba, Jamaica, Trinidad, and British Guiana. Other investigations were carried on in eastern Cuba in 1908, 1909, and 1910, and some observations were made in Porto Rico in 1910 and 1911.

(7) The location of the malady in the tissues with reference to the general structure of the tree makes it a particularly difficult one with which to experiment by direct means.

(8) Infection experiments with bacteria were successful in producing typical bud-rot. Infection experiments with fungi produced only dry and brown spots of limited extent.

(9) Experimental application of various approved fungicidal mixtures as remedies gave negative results. In general, those planters attending to ordinary methods of sanitation in their groves had little trouble with this disease.

(10) The cause of the disease in eastern Cuba is shown by repeated inoculation experiments to be a bacterial organism.

(11) Cultural studies of the organism causing the bud-rot show it to be practically identical with *Bacillus coli* (Escherich) Migula.

(12) Inoculations into coconut seedlings with *Bacillus coli* of animal origin give infections similar to inoculations with the coconut organisms.

(13) Comparison of *Bacillus coli* with the organisms first isolated by the writer and those isolated by at least one other worker indicate that *Bacillus coli* has been isolated in other experiments but not identified.

(14) Comparison of the bud-rot caused by *Bacillus coli* with several diseases of the coconut palm ascribed in literature to other causes indicate that several of these diseases are identical with bud-rot.

(15) Comparison of the bud-rot of the coconut palm with diseases of several other palms suggests that they may be the same thing.

(16) Studies with the microscope show that the bacteria thrive only in the meristematic tissues which are little or not at all lignified. Stomatal infections are common on the young tissues.

(17) It is believed that birds and insects are carriers of this disease, but the subject requires further study.

(18) The value of the annual importation of coconuts and coconut products into the United States is about $4,000,000. In 1906 Baracoa, Cuba, was our largest source of coconuts, with Trinidad as second.

RECOMMENDATIONS.

Owing to the widespread distribution of the bud-rot no coconut district in the American Tropics is secure from danger of infection. This bud-rot is due to a bacterial organism which may be distributed from place to place on the green unhusked coconut, and may be carried to healthy trees by insects or other animal life infesting diseased trees.

It is recommended, therefore, to cut down all badly diseased trees, or at least trim the tops and set fire to them. All débris, fallen leaves, nuts, etc., should be removed so as to destroy any infected material and any breeding place for insects which might serve to transmit the disease.

These ordinary methods of sanitation, together with proper methods of cultivation, if carried out faithfully by the planters of a whole district will reduce the loss by this disease to a minimum.

It is further urged that attempts be made to extend the coconut industry in Porto Rico to take the place of the rapidly failing groves of eastern Cuba. Inasmuch as this island appears to be free from the disease, it is urged also that special efforts be made by the General Government of the United States to keep it so.

INDEX.

WELLCOME LIBRARY INSTITUTE